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Sanacore® GM supplemented feed boosts the haemato-biochemical indices, immune response, anti-oxidative profile, and defense against *Aeromonas Vernoii* in Nile tilapia (*Oreochromis niloticus*).

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ABSTRACT

The present research was carried out to evaluate the effect of Sanacore® GM feed additives on the growth rate, hematological indices, biochemical indicators, anti-oxidative profile, non-specific immune response, and defense of Nile tilapia against *Aeromonas veronii* (*A. veronii*). Two fish groups of mean initial weight (13.5 ± 1.5g) were randomly allocated in triplicate and fed Sanacore® GM at a dose of 0 (control) and 5 g/kg diet for a period of 30 days. Fish were challenged by *A. veronii* at the experimental endpoint and the mortality rate was recorded for 15 days. Fish fed Sanacore® GM supplemented diet revealed significant increase in final body weight, haemato-biochemical indices (red blood cells, white blood cells, hemoglobin concentration, lymphocytes, neutrophils, serum total protein, and albumin levels), and immunological parameters (phagocytic activity, nitric oxide, lysozyme activity, IgM, and serum bactericidal activity). In addition, the anti-oxidative enzymes activity (superoxide dismutase and glutathione peroxidase) showed meaningful increase in Nile tilapia received Sanacore® GM incorporated diet. However, catalase enzyme displayed no significant activity ($P > 0.05$) in the supplemented group compared to the control group. The relative percentage survival was 100% in the challenged Sanacore® GM group compared the control. Our finding suggested that Sanacore® GM might considerably boost final growth rate, haemato-biochemical indices, immune response, anti-oxidative capability of Nile tilapia, as well as disease resistant against pathogenic strains of *A. veronii*.

1. INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is the most significant cultured freshwater species worldwide. Egypt was ranked the third largest tilapia producer after China and Indonesia (Nasr Allah et al., 2020). The high fish intensification exaggerates fish stressors and heightens the susceptibility of fish to infectious diseases (Wang et al., 2015). Bacterial diseases are a major impediment to the production in freshwater and marine fish, preventing many countries from developing economically (Subasinghe et al., 2001).

Motile Aeromonads, particularly *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas veronii* are the most important pathogenic bacteria of freshwater fishes causing a disease called motile Aeromonas septicemia (Youssuf et al., 2020a). It causes huge mortalities and threatens the sustainability of global tilapia production (El-Gohary et al., 2020). Fish producers frequently use antibiotics as treatment strategies to overcome mass losses in farms, this led to establishment of antibiotic-resistant bacterial strains (Youssuf et al., 2020b). Therefore, dietary supplementations of several nutraceutical compounds as an alternative to antibiotics have been used due to their enhancement of fish health status and resistance against fish pathogens (Soror et al., 2021).

Quorum sensing (QS) is a mechanism allowing microorganisms to communicate, which provides a way for pathogenic bacteria to reduce host immune response through the expression of virulence factors biofilm formation, and resistance to different antimicrobial agents (Nazzaro et al., 2019). Recently, an immunotherapeutic approach to quorum quenching has been presented as an alternative way to prevent bacterial infections (Fleitas Martínez et al., 2019). Sanacore®GM is one of the commercial feed additives that contains vegetable fatty acids, inactivated yeast and yeast extracts (*Saccharomyces cerevisiae*), and multiple herbal extracts promote fish immunity due to its quorum quenching effect. Therefore, the goal of the current study was to assess the inclusion effect of Sanacore®GM as quorum quenching on the growth, haemato-biochemical indices, immune response, anti-oxidative activity, and disease resistance of Nile tilapia.

2. MATERIAL AND METHODS

2.1. Feed additive and experimental diets

The feed additive Sanacore® GM (Nutriad International N.V.) was kindly supplemented by EgaVet Company, Egypt. The company supplemented the diet with a dose of 5 g/kg. The company's pilot studies recommended this dose based on preliminary work. An appropriate amount

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of distilled water (DW) containing the required dose of feed additive was added to 1 kg of ground commercial diet (Koudijs, Kapo Feed, Alexandria, Egypt; 30% crude protein) to produce a stiff paste. The paste was re-pelleted using a meat mincer and dried at room temperature (28 °C) for 48 hours. The medicated diet was packaged and stored at 4 °C.

2.2. Fish culture and feeding experiment

This research was carried out following the guidelines of the Committee of Animal Welfare and Research Ethics of Benha University, Faculty of Veterinary Medicine Egypt, with the permit number (BUFVTM-06-07-20).

Healthy Nile tilapia (13.5 ± 1.5g) was purchased from a fish farm in Kafr Elsheikh Governorate, Egypt. According to Austin and Austin (2007), fish were examined to confirm that they were free from diseases. Three hundred fish were allocated into six fiberglass tanks filled with 750 L of dechlorinated tap water (n = 50 per tank). Two experimental groups were settled in triplicate. The first control group received the normal basal diet, and the second group was fed a supplemented diet with Sanacore®GM (5 g/kg diet). All groups were fed 4% of their body weight twice daily for 30 days. The water temperature, dissolved oxygen, ammonia concentration, and pH were adjusted at 26±2°C, 6.0±0.5 mg/L, 0.53±0.07 mg/L, and 7.0±0.2, respectively. The exchange of water was done three times weekly to throw away fish feces and unconsumed food to maintain water quality parameters.

2.3. Blood and tissue sampling

After 30 days of feeding, three fish per tank were anesthetized with MS-222 (100 mg/L), and blood samples were collected using a plastic syringe moistened with EDTA for hematological and immunological studies. Another blood sample without an anticoagulant was obtained for collecting serum. The clotted blood was kept overnight in a refrigerator at 4 °C and centrifuged at 3000 rpm for 10 minutes to obtain sera, which were collected, pooled, and kept at -80°C for subsequent biochemical analysis.

2.4. Haemato-biochemical pattern

Blood elements (RBCs and WBCs) were counted with a Neubauer hemocytometer, according to Kanaev (1985). Blood hemoglobin concentration (Hb) (g/dl) was determined using commercial colorimetric kits (Diamond Diagnostic, Egypt). The differential leukocyte counts (neutrophil, lymphocyte, and monocyte) were counted using blood smears stained with Giemsa, according to Stoskopf (1993).

Total protein and albumin levels in serum samples were assessed by the method of Lowry et al. (1951). The globulin levels were calculated by subtracting the albumin value from the total serum protein.

2.5. Anti-oxidative enzyme assays

Liver samples (one gram) from all supplemented and control groups were cut and rinsed with phosphate-buffered saline (PBS) (pH 7.4). Then samples were homogenized in 9 ml of cooled PBS (pH 7.4) using an electrical homogenizer (Heidolph, Germany) and centrifuged at 4000 ×g/15 min/4 °C. The resultant supernatants were stored at -20 °C for the determination of antioxidant enzymes. Superoxide dismutase activity assay (SOD), catalase activity assay (CAT), and glutathione peroxidase (GPx) were performed using commercial kits (Bio-diagnostic, Egypt).

2.6. Immunological assays

Phagocytic activity was measured following the protocol reported by Abu-Elala and Ragaa (2015). Serum bactericidal and lysozyme activities were assayed according to El-Asely et al. (2014) and Schultz (1987), respectively. Serum IgM level was measured spectrophotometrically following the protocol of ELISA kits (Cusabio Biotech Co. Ltd., USA), and serum nitric oxide was estimated after following Bio-diagnostic commercial kit instructions.

2.7. Challenge test

The pathogenic strains of *Aeromonas veronii (A(HY2*) and A(HY4)) were previously isolated from diseased Nile tilapia and well identified, verified, and tested for pathogenicity (Youssuf et al., 2020a). The bacterial cell concentration (1.8×10^8 cells/ml) was prepared and adjusted as described by Youssuf et al. (2020a).

After sample collection, 150 fish in supplemented and control groups were distributed into fifteen well-prepared glass aquaria (30×40×90 cm) and designed into five groups in triplicate (10 fish per replicate, N = 30 per group). The first and second supplemented groups were injected in the peritoneal cavity with 0.2 ml of *A. veronii* (HY2) and *A. veronii* (HY4) bacterial suspension (1.8×10^8 cells/ml), respectively. The third and fourth control groups were injected as previous groups and served as control positives. The last fifth group was kept as a control negative (non-challenged group). During the challenge test, all groups continued to receive their prescribed diets; any abnormal signs in the fish were observed, and mortality was recorded daily for 15 days for the determination of relative percentage survival (RPS) following Amend (1981).

2.8. Statistical analysis

The obtained records were tested by an independent sample. The difference is using SPSS software V.16 (SPSS, Richmond, USA). The difference among means was regarded as statistically significant at $P< 0.05$. The data was displayed as mean± standard error.

3. RESULTS

3.1. Growth and hemo-biochemical indices

The Sanacore® GM supplemented group revealed significant increases ($P< 0.05$) in the final weight (21.92±0.47 g) compared to the control (47.49 ± 1.77g). There was a significant increase ($P < 0.05$) in RBCs, WBCs, lymphocytes, neutrophils, and Hb concentrations in the supplemented group compared to the control group (Table 1). In the same line, serum

total protein and albumin showed significant ($P< 0.05$) increases in the Sanacore®GM-fed group compared to the control, with no significant changes in globulin value (Fig. 1).

Table. 1: Hematological parameters of *O. niloticus* fed Sanacore® GM for 30 days.

Group	RBCs $\times10^6/\mu l$	WBCs $\times10^3/\mu l$	HB (g/dl)	Neutrophil	Lymphocyte	Monocyte
Control	$2.35^b\pm0.15$	$2.69^b\pm0.81$	$7.49^b\pm0.36$	$44.35^b\pm3.06$	$24.60^b\pm0.68$	$4.57^a\pm0.22$
Sanacore® GM	$3.65^a\pm0.17$	$6.56^a\pm1.47$	$10.36^a\pm0.82$	$58.92^a\pm0.89$	$35.39^a\pm1.98$	$5.16^a\pm0.06$

Values are presented as mean (n= 3) ± standard error. The mean values for each parameter in the same column with a distinct superscript letter were substantially different between groups ($P< 0.05$). RBCs; red blood cell counts, WBCs; white blood cell counts, Hb; hemoglobin concentration.

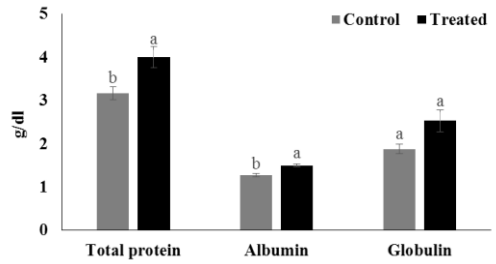

Fig 1: Serum total protein, albumin and globulin levels of Nile tilapia fed Sanacore® GM for 30 days. Values are mean ± SEM. Mean values with different superscript letter were significantly different between groups ($P< 0.05$).

3.2. Anti-oxidative enzyme activity

SOD and GPX activities of the group fed a Sanacore® GM-supplemented diet revealed a significant increase (51.88±5.19 U/g; 50.66±3.76 U/g) compared to the control (27.11±4.27 U/g; 28.74±4.16 U/g), respectively. Meanwhile, the CAT enzyme recorded no significant activity ($P> 0.05$) among the supplemented (41.26±1.32 U/g) and control groups (40.51±5.77 U/g) (Fig 2).

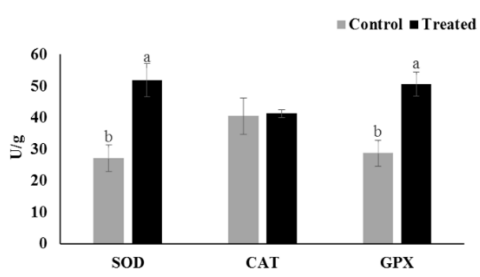

Fig2:Antioxidant enzymes [superoxide dismutase (SOD)and Catalase (CAT), glutathione peroxidase (GPX)]in Nile tilapia fed Sanacore® GM for 30 days. Values are mean ± SEM. Mean values with different superscript letter were significantly different between groups (P< 0.05).

3.3. Immunological parameters

Phagocytic activity and Nitric Oxide concentration were significantly increased ($P< 0.05$) in the supplemented group (Fig. 3). Moreover, dietary Sanacore® GM enhanced serum lysozyme activity and IgM level as compared with those receiving a control basal diet (Fig. 4). In addition, serum bactericidal activity against the pathogenic *A. veronii* strains A(HY2) and A(HY4) was significantly enhanced in the supplemented group compared to the control ($P< 0.05$) (Fig 5).

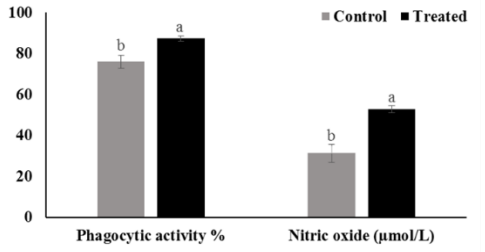

Fig 3: Phagocytic activity and nitric oxide level in Nile tilapia fed Sanacore® GM for 30 days. Values are mean ± SEM. Mean values with different superscript letter were significantly different between groups (P< 0.05).

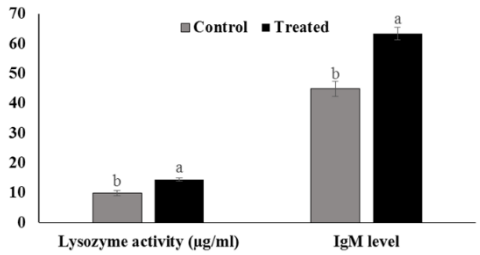

Fig4: Serum lysozyme activity and IgM level in Nile tilapia fed Sanacore® GM for 30 days.Values are mean ± SEM. Mean values with different superscript letter were significantly different between groups (P< 0.05).

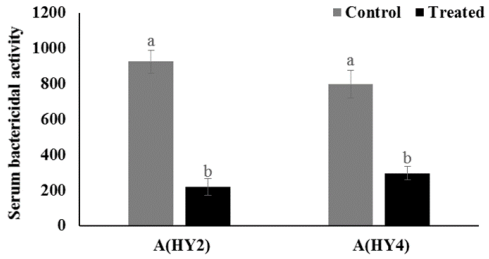

Fig 5: Serum bactericidal activity against A.veronii strains (HY2) and (HY4)in Nile tilapia fed Sanacore® GM for 30 days. Values are mean ± SEM. Mean values with different superscript letter were significantly different between groups (P< 0.05).

3.4. Challenge Test

No mortality was recorded in Sanacore®GM-fed groups challenged with the two *A. veronii* strains. On the other hand, the mortality rates in the challenged control group with *A. veronii* strains (A (HY2) or A (HY4)) reached 100% and 90%, respectively. It was noticed that the supplemented challenged group didn't exhibit any signs of septicemia (loss of appetite, erratic movement, fin erosion, abdominal dropsy, and exophthalmia), which were clear in the challenged control group.

4. DISCUSSION

Immunomodulators have lately become more popular in aquaculture due to their environmental friendliness and low cost, making them a possible alternative to antibiotics, vaccines, and chemicals (Zahran et al., 2018). The feed additive Sanacore® GM supplementation improved Nile tilapia's final weight. This improvement could be attributed to the presence of yeast extract and herbal substances, which are proven to enhance fish palatability, and gut morphometry, and stimulate the production of digestive enzymes (Li and Gatlin, 2006; Abdel-Tawwab et al., 2020). Similarly, Abdel-Tawwab et al (2022) and Eissa et al (2023) reported that the supplementation of Sanacore®GM significantly improved the final body weight of Pacific white shrimp (*Litopenaeus vannamei*) and gilthead seabream (*Sparus aurata*) respectively.

Fish hematological parameters are a useful tool for assessing health in response to feed supplements (Ayyat et al., 2018). WBCs play a major role in fish innate immunity and are studied as a marker of fish health conditions (Mohammed et al., 2020). In the current study, the hematological parameters (RBCs, WBCs, and Hb concentration, lymphocytes, and neutrophil count) of Nile tilapia were significantly increased with Sanacore® GM feed additive, which indicates that Sanacore®GM has immuno-stimulatory and anti-infection characteristics. Feeding gilthead seabream on Sanacore® GM for ten weeks significantly increased RBCs count and Hb level (Abdel-Tawwab et al., 2022).

Serum total protein and globulin levels are strong indicators of the innate immunity response of fish (Hoseini et al., 2018). In the present study, the supplemented group had a significant increase in serum total protein and albumin levels. This result comes in accordance with data obtained in gilthead seabream fed 0.4% Sanacore® GM (Abdel-Tawwab et al., 2022). The improved protein level may be attributed to high WBCs as a major source of protein (Misra et al., 2006).

Antioxidant enzymes are crucial factors of defense in fish, which provide protection for aquatic animals against the stressful environment (Abd El-Gawad et al., 2019). SOD and CAT enzymes are the first antioxidant scavengers of superoxide radicals and protect the tissue from free radicals (Zhang et al., 2012). In this research, liver SOD and GPX activities showed a big rise in fish that were fed a diet that included Sanacore® GM. However, CAT activity recorded a non-significant difference between the two groups. Abdel-Tawwab et al. (2023) also recorded high SOD and CAT activities in gilthead seabream fed Sanacore® GM supplemented diet.

Fish lysozyme is generated from leukocytes and is considered one of fish's innate immunity components that acts as a protective barrier against bacterial infections by preventing biofilm formation (Misra et al., 2006). Also, serum bactericidal activity is an important tool used to evaluate the host defense mechanism against bacteria (Biller-Takahashi et al., 2012). Dietary supplementation of Sanacore® GM, in the current study, improved immune parameters (phagocytic activity, nitric oxide levels, lysozyme activity, IgM and bactericidal activity). These findings are in accordance with results obtained by Abdel-Tawwab et al. (2023) in gilthead seabream and Pacific white shrimp (Eissa et al., 2023). The highest phagocytic activity could be attributable to macrophage activation or an increase in lysozyme level (Laith et al., 2017).

The current study revealed that Nile tilapia fed Sanacore® GM, showed 100 % RPS following a challenge with *A. veronii*. This could be because of its ingredients, such as inactivated S. cerevisiae, which helped boost immunity and balance healthy gut bacteria (Abdel-Tawwab, 2012), or because Sanacore® GM has a property that stops quorums. Sanacore® GM administration at different levels in Nile tilapia (Favero et al., 2020), and gilthead seabream (Palenzuela et al., 2020; Abdel-Tawwab et al., 2023) augments fish resistance against francisellosis, streptococcosis, enteromyxosis and vibriosis. Furthermore, studies have shown that incorporating various herbal plants into fish diets improves fish resistance to bacterial infection (Soror et al., 2021; Abd El-Latif et al., 2021; Abd El-Naby et al., 2023).

5. CONCLUSIONS

In conclusion, the dietary inclusion of a functional feed additive (Sanacore® GM) at a dose of 5 g/kg diet has a positive effect on growth, hematological, and immunological responses, antioxidant status, and disease resistance in Nile tilapia. In addition, it has an anti-QS mechanism, thereby; it could be used as an appropriate supplement in the diet of Nile tilapia.

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Declaration of Conflict of Interest

The authors declare that they have no conflict of interest about this manuscript.

6. REFERENCES

1. Abd El-latif, A.M., Abd El-Gawad, E. A., Soror E. I., Shourbela R. M., Zahran E.2021. Dietary supplementation with miswak (*Salvadora persica*) improves the health status of Nile tilapia and protects against *Aeromonas hydrophila* infection. Aquaculture Report, 19. https://doi.org/10.1016/j.aqrep.2021.100594
2. Abd El-Naby, A. S., El Asely A.M., Hussein M.N., Fawzy R.M., Abdel-Tawwab M. 2023. Stimulatory effects of dietary chia (*Salvia hispanica*) seeds on performance, antioxidant-immune indices, histopathological architecture, and disease resistance of Nile tilapia.

Aquaculture,563.https://ui.adsabs.harvard.edu/link_gateway/2023Aquac.56338889A/doi:10.1016/j.

3. Abdel-Tawwab, M. 2012. Interactive effects of dietary protein and live bakery yeast, *Saccharomyces cerevisiae* on growth performance of Nile tilapia, *Oreochromis niloticus* (L.) fry and their challenge against *Aeromonas hydrophila* infection. Aquaculture International 20, 317–331.
4. Abdel-Tawwab, M., Abo Selema, T.A.M., Abotaleb, M.M., Khalil, R.H., Sabry, N.M., Soliman, A. M., Eldessouki, E.A.A. 2023. Effects of a commercial feed additive (Sanacore® GM) on immune-antioxidantprofile and resistance of gilthead seabream (*Sparus aurata*) against *Vibrio alginolyticus* infection. Annals of Animal Science, 23 (1), 185–193
5. Abdel-Tawwab, M., Abo Selema,T.A.M., Soliman, A.M., Shady, S.H.H., Khalil, R.H. 2022.Effects of a commercial feed additive (Sanacore® GM) on water quality, productive performance and blood profile of gilthead seabream (*Sparus aurata*). Aquaculture Research, 53, 6310–6323.
6. Abdel-Tawwab, M., Adeshina, I., Issa, Z.A. 2020. Antioxidants and immune responses, resistance to *Aspergilus flavus* infection, and growth performance of Nile tilapia, *Oreochromis niloticus*, fed diets supplemented with yeast, *Saccharomyces serevisiae*. Animal Feed Science and Technology 263. https://doi.org/10.1016/j.anifeedsci.2020.114484
7. Abu-Elala, N.M., Ragaa, N.M. 2015. Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*. Journal of Advanced Research, 6 (4), 621–629.
8. Amend, D.F. 1981. Potency testing of fish vaccines. In: Anderson DP, HennessenH, editors. Fish biologics: sero diagnostics and vaccines. Developments in biological standardisation. Basel: Karger; p. 447-54.
9. Ameur, W.B., De Lapuente, J., El Megdiche, Y., Barhoumi, B., Trabelsi, S., Camps, L., Serret, J., Ramos-López, D., Gonzalez-Linares, J., Driss, M.R. 2012. Oxidative stress, genotoxicity and histopathology biomarker responses in mullet (*Mugil cephalu*s) and sea bass (*Dicentrarchus labrax*) liver from Bizerte Lagoon (Tunisia). Marine Pollution Bulletin 64, 241–251.
10. Austin, B., Austin, D.A 2007. Bacterial fish pathogens, Disease of farmed and wild fish, 4th Ed. Springer Praxis, Godalming.
11. Ayyat, M.S., Ayyat, A.M.N., Al-Sagheer, A.A., El-Hais, A.M.2018. Effect of some safe feed additives on growth performance, blood biochemistry, and bioaccumulation of aflatoxin residues of Nile tilapia fed aflatoxin-B1 contaminated diet. Aquaculture. 495, 27–34.
12. Biller-Takahashi, J.D., Takahashi, L.S., Marzocchi-Machado, C.M., Zanuzzo, F.S., Sabioni, R.E., Urbinati, E.C.2012. Hemolytic activity of alternative complement pathway as an indicator of innate immunity in pacu (*Piaractus mesopotamicus*). Revista Brasileira de Zootecnia, 41, 237–241.
13. Bo Liu, X., Yanhui, H., Jun, P., Xigin, Q., Ruli,C. 2010. Effects of anthraquinones extracted from Rheum *officinale* Bail on the growth, the nonspecific immune response of *Macrobrachium rosenbergii*. Aquaculture, 3 (10) :13-19.
14. Eissa, E.H., Elbahnaswy, S., El-Baz, A.H., El-Haroun, E., Ashour, M., Mansour, A.T., Saadony, S., El-Saeed, A.F., Kabary, H., Jastaniah, S.D., Alaidaroos, B. A.E., Shafi M., Abd El-Hack, M.E., Eissa, M.E.H., Eldessouki, E.A. 2023 Effects of dietary commercial phytobiotic "Sanacore® GM" on Pacific white shrimp (*Litopenaeus vannamei*) growth, immune response, redux status, intestinal health, and disease resistance against *Fusarium solani*. Aquaculture International. https://doi.org/10.1007/s10499-023-01310-5
15. El-Asely, A.M., Abbass, A.A., Austin,B. (2014). Honeybee pollen improves growth, immunity and protection of Nile tilapia (*Oreochromis niloticus*) against infection with *Aeromonas hydrophila*. Fish & Shellfish Immunology; 40:500-506.
16. El-Gohary, F.A., Zahran, E., Abd El-Gawad, E.A., El-Gohary, A.H., Abdelhamid, F.M., El-Mleeh, A., Elmahallawy, E.K., Elsayed, M.M. 2020. Investigation of the Prevalence, Virulence Genes, and Antibiogram of Motile Aeromonads Isolated from Nile Tilapia Fish Farms in Egypt and Assessment of their Water Quality. Animals, 10(8), 1432. https://doi.org/10.3390%2Fani10081432
17. Favero, L.M., Nuez-Ortín, W.G., Isern-Subich, M.-M., de Padua Pereira, U. 2020. Use of a functional feed additive to reduce mortality from franciselosis and streptococcosis. Aquafeed: Adv. Proces. Form., 12, 23–26.
18. Fleitas Martínez, O., Rigueiras, P.O., Pires, Á.D.S., Porto, W.F., Silva O.N., De la Fuente-Nunez, C., Franco, O.L. 2019. Interference with quorum-sensing signal biosynthesis as a promising therapeutic strategy against multidrug-resistant pathogens. Frontiers in Cellular Infection and Microbiology, 8, 444. https://doi.org/10.3389/fcimb.2018.00444
19. Hoseini, S.M., Hoseinifar, S.H., Doan, H.V. 2018. Effect of dietary eucalyptol on stress markers, enzyme activities and immune indicators in serum and haematological characteristics of common carp (*Cyprinus carpio*) exposed to toxic concentration of ambient copper. Aquaculture Research, 49, 3045–3054.
20. Kanaev, A. F. 1985.Veterinary Hygiene in Fish Farming Moscow, 140-194.
21. Laird, L.M., Needham, T. 1988. Growth, nutrition and feeding, Salmon and trout farming, England, Ellis Horwood Limited, 202-216.
22. Laith, A.A., Mazlan, A.G., Effendy, A.W., Ambak, M.A., Nurhafizah, W.W.I., Alia, A.S., Jabar, A., Najiah, M. 2017. Effect of *Excoecaria agallocha* on non-specific immune responses and disease resistance of *Oreochromis niloticus* against *Streptococcus agalactiae*. Research in Veterinary Science, 112, 192–200.
23. Li, P., Gatlin, D.M. 2006. Nucleotide nutrition in fish: Current knowledge and future applications. Aquaculture, 251, 141– 152.
24. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with thefolin phenol reagent. J.Biol.Chem., 1951; 193: 265-275.Oser B L. Hawk's physiological chemistry. 14 ed., Tata McGraw Hill Publishing Co.Ltd., New Delhi, 1965
25. Misra, C.K., Das, B.K., Mukherjee, S.C and Pattnaik, P.2006. Effect of multiple injections of b-glucan on non-specific immune response and disease resistance in *Labeo rohita* fingerlings, Fish &Shellfish Immunology, 20, 305-19.
26. Mohammed, E., Kamel, M., El Iraqi, K., Tawfik, A.M., Khattab, M.S.,Elsabagh, M. 2020.*Zingiber officinale* and *Glycyrrhiza glabra*, individually

or in combination, reduce heavy metal accumulation and improve growth performance and immune status in Nile tilapia, *Oreochromis niloticus*. Aquaculture Research 51: 1933-1941.

27. Nasr Allah, A., Gasparatos, A., Karanja, A., Dompreh, E.B., Murphy, S. , Rossignoli, C.M. , Phillips, M. , Charo-Karisa, H. 2020. Employment generation in the Egyptian aquaculture value chain: implications for meeting the sustainable development goals (SDGs). Aquaculture, 520. https://doi.org/ 10.1016/j.aquaculture.2020.
28. Nazzaro, F., Fratianni, F., d'Acierno, A., De Feo, V., Ayala-Zavala, F.J Gomes-Cruz, A. 2019. "Effect of polyphenols on microbial cell-cell communications," in Quorum Sensing: Molecular Mechanism and Biotechnological Application, ed. G.Tommonaro (Cambridge, MA: Academic Press), 195–223.
29. Palenzuela, O., Del Pozo, R., Piazzon, M.C., Isern-Subich, M.M., Ceulemans, S., Coutteau, P., Sitjà-Bobadilla, A. 2020.Effect of a functional feed additive on mitigation of experimentally induced gilthead sea bream *Sparus aurata* enteromyxosis. Disease Aquatic Organisms 138: 111–120.
30. Sánchez-Martínez, J.G., Rábago-Castro, J.L., Vázquez-Sauceda, M.D.L.L., Pérez- Castañeda, R., Blanco-Martínez, Z& Benavides-González., F. 2017. Effect of β-glucan dietary levels on immune response and hematology of channel catfish *Ictalurus punctatus* juveniles. Latin American Journal of Aquatic Research, 45 ,4, 690–698.
31. Schultz, L.A. 1987. Methods in clinical chemistry. The C.V. Mosby Co. St. Louis, pp. 742-746.
32. Soleimany, V., Banaee, M., Mohiseni, M., NematdoostHagi, B., Mousavi Dehmourdi, L. 2016. Evaluation of pre-clinical safety and toxicology of *Althaea officinalis* extracts as naturopathic medicine for common carp (*Cyprinus carpio*). Iranian journal of Fisheries Sciences 15,2: 613-629.
33. Soror, E.I., El-Asely, A.M., Abdel-Gawad, E.A., Radwan, H.A., Abbass, A.A. 2021. Recuperative effects of honeybee pollen, ginger (*Zingiber officinale*), and *Moringa oleifera* in Nile tilapia (*Oreochromis niloticus* L.) after sub-lethal exposure to dimethoate. Aquaculture 530. https://doi.org/10.1016/j.aquaculture.2020.735886
34. Subasinghe, R.P., Bondad-Reantaso, M.G& McGladdery, S.E. 2001. Aquaculture development, health and wealth. In: Subasinghe, R.P., Bueno, P., Phillips, M.J., Hough, C., McGladdery, S.E., Arthur, J.R. (Eds.), Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20–25 February 2000. NACA, Bangkok and FAO, Rome, 167–191.
35. Wang, J.L., Meng, X.L., Lu, R.H., Wu, C., Luo, Y.T, Yan, X., Li, X.J., Kong, X.H& Nie, G.X. 2015. Effects of *Rehmannia glutinosa* on growth performance, immunological parameters and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio* L.). Aquaculture, 435, 293–300.
36. Woldehiwet, Z., Rowan, T.G. 1990. Some observations on the effects of age of calves on the phagocytosis and killing of *Staphylococcus aureus* by polymorphonuclear leucocytes Br. Vet. J. 146, 2:165-70.
37. Youssuf, H., Abdel Gawad, E.A., El Asely, A.M., Elabd, H., Matter, A.F, Shaheen, A.A., Abbass, A.A. 2020a. Insight into summer mortalities syndrome in farmed Nile tilapia (*Oreochromis niloticus*) associated with bacterial infections. Benha Veterinary Medical Journal, 39 ,1, 111-118.
38. Youssuf, H., El-Asely, A.M., Abdel Gawad, E.A., Abbass, A.A., Shaheen, A.A. 2020b. Control of some septicemic bacterial infections in *Oreochromis niloticus*, with special reference to their antimicrobial resistant genes. Benha Veterinary Medical Journal 39 , 2, 40-46.
39. Zahran, E., Abd El-Gawad, E.A., Risha, E.2018. Dietary *Withania somnifera* root confers protective and immunotherapeutic effects against *Aeromonas hydrophila* infection in Nile tilapia (Oreochromis niloticus). Fish &Shellfish Immunology, 80, 641–650.
40. Zhang, J., Liu, Y., Tian, L., Yang, H., Liang, G., Xu, D. 2012. Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol. 3, 1027–1032.