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

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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 x 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 Raga (2015). Serum bactericidal and lysozyme activities were assayed according to El-Asely et al. (2014) and Schulz (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 x 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 (30x40x90 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 x 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 hemato-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.

<table>
<thead>
<tr>
<th>Group</th>
<th>RBCs (\times10^6/\mu l)</th>
<th>WBCs (\times10^3/\mu l)</th>
<th>Hb (g/dl)</th>
<th>Neutrophil</th>
<th>Lymphocyte</th>
<th>Monocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.33(\pm)0.15</td>
<td>2.69(\pm)0.81</td>
<td>7.49(\pm)0.36</td>
<td>44.33(\pm)3.06</td>
<td>24.60(\pm)0.68</td>
<td>4.57(\pm)0.22</td>
</tr>
<tr>
<td>Sanacore® GM</td>
<td>3.65(\pm)0.17</td>
<td>6.56(\pm)1.47</td>
<td>10.36(\pm)0.82</td>
<td>58.92(\pm)0.89</td>
<td>35.39(\pm)1.98</td>
<td>5.16(\pm)0.06</td>
</tr>
</tbody>
</table>

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.

3.2. Anti-oxidative enzyme activity
SOD and GPX activities of the group fed a Sanacore® GM-supplemented diet revealed a significant increase \((51.88\pm5.19\ \text{U/g};\ 50.66\pm3.76\ \text{U/g})\) compared to the control \((27.11\pm4.27\ \text{U/g};\ 28.74\pm4.16\ \text{U/g})\), respectively. Meanwhile, the CAT enzyme recorded no significant activity \((P>0.05)\) among the supplemented \((41.26\pm1.32\ \text{U/g})\) and control groups \((40.51\pm5.77\ \text{U/g})\) (Fig 2).

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).

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 \((HY2)\) and \((HY4)\) in \(O.\) niloticus fed Sanacore® GM for 30 days. Values are mean ± SEM. Mean values with different superscript letter were significantly different between groups \((P<0.05)\).
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 immunostimulatory 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., 2020). 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 (Missra 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


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


