Probiotic Zado® (*Ruminococcus Flavefaciens*) boosts hematology, immune, serum proteins, and growth profiles in Nile tilapia (*Oreochromis niloticus*)

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**ARTICLE INFO**

**Keywords:**
- *Oreochromis niloticus*
- Zado®
- Immunity
- Biochemical
- Growth parameters

**ABSTRACT**

Probiotics application in aquaculture could be a key solution to enhance the overall immune and growth indicators of cultured fish. Several bacteria have demonstrated encouraging results as probiotics for fish. The current study evaluated the possible effects of Zado® (*Ruminococcus Flavefaciens* 28 × 10⁸ CFU) dietary incorporation at 1 and 2 g/kg diet for 6 weeks on growth, hematological profile, immune performance, the biochemical, and anti-oxidative profiles of *Oreochromis niloticus*. Sampling was performed at the end of the third and sixth week. Fish fed with Zado® enriched diets showed (P < 0.05) significantly improved hematologic (MCHC, MCH, MCV, and PCV and RBCs count) and leukocytic readings (WBCs, monocytes and lymphocytes). The immune (phagocytosis, lysozyme U/ml, and nitric oxide pmol/ml) parameters were (P < 0.05) markedly increased in Zado® incorporated groups. Biochemical parameters (globulin, albumin and total proteins; AST and ALT) levels showed significant (P < 0.05) improvement at three and six weeks in Zado® groups. Serum glucose concentration was significantly higher in Zado® groups at six weeks, while was only higher for 2 g/kg Zado® at six weeks. Also, cortisol level was lower in both Zado® groups at three weeks, while was only lower for 1 g/kg Zado® at six weeks. In addition, antioxidants Gpx, SOD, and CAT were (P < 0.05) significantly higher in Zado® treatments, while pro-oxidant MDA was (P < 0.05) significantly decreased. Moreover, growth performance was also (P < 0.05) markedly boosted in Zado® incorporated groups compared to the control. Conclusively, our results demonstrated that Zado® probiotic is a safe alternative for *O. niloticus* with beneficial effects on hematological parameters, immune, biochemical, antioxidants, and growth profiles.

1. Introduction

The intensification of aquaculture production is usually challenged by infections including bacterial and parasitic burdens, which obligates the usage of chemicals and antibiotics to control disease outbreaks [18]. The injudicious use of antibiotics inevitably led to the progress of resistance, mutagenic microbial strains, and detrimental effects on fish and consumer health [14, 25]. Therefore, it is imperative to find alternative eco-friendly sources as prebiotics and probiotics which can improve fish health, performance, and immunity without side effects to the fish themselves or the consumer health [7, 27, 32]. Probiotics were scientifically defined as reviewed in [9] as live microbes which if supplied in ample amounts will benefit the host. Probiotics usage in aquaculture is a smart bio-friendly approach to reduce the effect of infectious diseases and to obtain beneficial effects on gut health but is still with a limited application [11, 26]. Nevertheless, applying various probiotic species (e.g., *Pseudomonas, Nitrobacter, Cellulomonas, and Enterobacter*) was not effective for channel catfish to enhance water quality; therefore, knowledge of the mode of action of probiotics in cleaning water is still in its early stages [12, 34]. The commercially available probiotics in aquaculture are limited [8].

To the best of our knowledge, no preceding studies have described the effect of *Ruminococcus Flavefaciens* on growth, immune and hematological profiles in Nile tilapia *O. niloticus*. Thus, the current study was conducted to evaluate the potential beneficial effects of Zado® probiotic dietary inclusion on hematobiochemical profiles, immunity, and anti-oxidant system in *O. niloticus*.

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https://doi.org/10.1016/j.fsirep.2021.100021

Received 2 July 2021; Received in revised form 18 August 2021; Accepted 27 August 2021

Available online 29 August 2021

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2. Material and methods

2.1. Fish and experimental conditions

The study was performed on a total of 270 O. niloticus (16.0 ± 0.5 g) procured and transported to (140 cm height × 140 cm diameter) fiberglass tanks at the fish production unit (1), Egyptian military veterinary administration, Egypt where the experiment was carried out. Fish were subjected to acclimation for additional 10 days. Water temperature, oxygen and pH were 26.0 ± 0.5°C and dissolved oxygen at 6.2±0.4 mg/L. All the experimental processes were performed following the Research Ethics Board, Faculty of Veterinary Medicine, Benha University BUPVTM 08-02-21.

2.2. Zado® probiotic and feeding trial

Zado® was obtained as a commercial product from bactizad®, Egypt containing (Ruminococcus Flavefaciens 28 × 10^8 CFU) gram-positive anaerobic bacteria. Basal diet (Dry matter 67.14, crude protein 30.08, crude fiber 8.28 and lipid 5.84) was inoculated with Zado® probiotic at a rate of 1 and 2 g/kg and prepared by the feed manufacture factory at the food industries and packing complex (Fipco), affiliated to the logistic authority of Egyptian armed forces following the manufacturer’s procedures.

Fish were housed in three groups (90 fish per group, in triplicates) and received 0, 1 and 2 g Zado® probiotic /kg diet for six weeks, twice daily at 3% of total fish weight (9 am and 4 pm).

2.3. Blood and tissue sampling

Nine fish from each group (three/replicate) were sampled for blood collection from caudal blood vessels using 1 cc U-100 plastic syringe (Ameco, Egypt) and divided into two parts. First part was added to EDTA-moistened 1.5 ml tubes and kept on crushed ice until centrifugation to evaluate immune (phagocytic index and activity); and hematological parameters including RBCs and WBCs counts, differential leukocytic count, Packed Cell Volume (PCV), Hemoglobin concentrations. The second part was added to tubes without anticoagulant centrifuged at 1372 × g for 15 min to collect serum for assessing serum protein (albumin, globulin, and total protein), immune (lysozyme and nitric oxide (NO)), and biochemical (AST, ALT, glucose, and cortisol levels) parameters.

Following blood sampling, fish were carefully dissected to collect liver samples. Each 100 mg of liver samples were rinsed with 1 ml phosphate buffered saline (PBS, pH 7.4) and homogenized in 50 mM PBS containing 1MmEDTA (pH 7.4) for assaying Superoxide dismutase (SOD), Malondialdehyde (MDA), glutathione peroxidase (Gpx), and catalase (CAT) after centrifugation at 15,000 × g for 10 min.

2.4. Hematological analysis

RBCs and WBCs were counted by using a hemocytometer and Schew’s solution according to [29], differential leukocyte count (DLC) was estimated per (Levine [17]) using Geimsa staining technique. PCV was measured through dividing the height of RBCs column in capillary tube after centrifugation by the total height of the blood column and then multiplied by 100 according to [29]. Hemoglobin (Hb) concentration was carried out using Drabkin’s reagent clorimetric kits (Diamond diagnostics, Egypt); and hemoglobin concentration readings (MCV, MCH, and MCHC) were measured following [6].

2.5. Immune parameters

Phagocytic index and activity were measured using 1 × 10^6 Aeromonas hydrophila bacterial strain supplied by Sakha Aquaculture Research Unit, Central Lab, Egypt; where 0.5 ml of blood was added to 0.25 ml of 1 × 10^6 Aeromonas hydrophila, mixed and incubated at 28°C for 30 min, and blood smears were stained by Giemsa/May-Grünwald according to [5]. The number of active engulfing leukocytes was counted as percentages to total leukocyte number and PI = phagocytized bacteria total number / phagocytizing cells number.

Lysozyme activity was measured through adding 25 µl of the undilated serum to 175 µl of the substrate solution (Micrococcus lysodeikticus lyophilized cells, Inova Biotechnology, China). Alterations in turbidity was recorded every 30 s [36] for 5 min at 450 nm using the microplate ELISA reader at 450 nm.

Nitric oxide (NO) was assayed at 450 nm through a commercial Kit (ab211083, Abcam, USA).

2.6. Biochemical parameters

The assay of serum albumin, globulin, and total protein was carried in triplicates according to the manufacturer’s instructions (RA-50 chemistry analyzer (Bayer) using readymade chemicals (kits) supplied by Spinreact Co. Spain) by measuring the absorbance of sample and standard against reagent blank spectrophotometrically at 550 nm wavelength according to company protocol.

ALT and AST; and glucose and cortisol concentrations were analyzed spectrophotometrically at 450 nm using kits supplied from (BioMed, Egypt) according to the kits’ working protocol.

2.7. Antioxidants’ profile

MDA was measured through recording TBA reactive product at 95°C for 30 min at 545 nm, SOD was assayed through estimating nitro blue tetrazolium at 550 nm, Gpx and CAT were measured at 530 nm and 510 nm, respectively using (BioMed, Egypt) commercial kits and according to a protocol developed by manufacturer.

2.8. Growth performance

Body weight for all fish was recorded at the start and end of the experiment, where:

- Specific Growth Rate % day⁻¹ = (ln final body mass - ln initial body mass) / (number of days) × 100
- Body Mass Gain % = 100 × (final body mass - initial body mass) / initial body mass;
- Length gain rate % = 100 × (Average terminal body length – Average initial body length) / (Average initial body length) × 100;
- Feed Conversion Ratio = F / (Wf – Wi);

For somatic indices: the weight of the organ was divided by the fish’s average body weight and multiplied by 100.

2.9. Statistical analysis

One-way ANOVA and Duncan’s multiple range tests were used to analyze the obtained data by SPSS statistical software (v. 22.0). Significance was considered at P-value < 0.05.

3. Results

3.1. Hematological profile

The average values of MCHC, MCH, MCV, PCV, and RBCs count were (P < 0.05) significantly higher in supplemented fish groups than in the control group at 3 weeks. At 6 weeks, 2 g/kg Zado® revealed the highest increase in RBCs and Hb readings (Table 1). Results of WBCs indices showed the same pattern of significance (1 g > 2 g > control) at both three and six weeks sampling times. WBCs values were (P < 0.05) significantly high in 1 g/kg Zado® group, while the percentage of monocytes and lymphocytes were significantly higher in 2 g/kg Zado®
3.2. Immune-related parameters

The phagocytic index and activity at three and six weeks were ($P < 0.05$) significantly higher in 1 and 2 g/kg Zado® supplemented groups than in the control group. Phagocytic activity increased in the supplemented groups at the end of week six, while it was stable in the control group (Fig. 1). At the end of the third week, serum levels of NO were higher in the 1 g/kg Zado® group, while at the end of week six, both 1 and 2 g/kg Zado®-supplemented groups revealed ($P < 0.05$) significant increase compared to the control (Fig. 2).

At the end of the third and sixth week, lysozyme activity was ($P < 0.05$) significantly higher in Zado®-supplemented groups, with the highest significant increase for 2 g/kg Zado® group compared to the control (Fig. 3).

3.3. Biochemical parameters

Throughout the entire experiment serum globulin, albumin and total protein results showed a ($P < 0.05$) significant improvement in Zado®-supplemented fish (Table 3).

Liver function enzymes AST and ALT were ($P < 0.05$) significantly lower in Zado®-supplemented groups at three and six weeks (Table 3).

Serum glucose concentration was ($P < 0.05$) significantly higher in Zado®-supplemented fish at three weeks, while was only higher for 2 g/kg Zado® group at six weeks. Also, cortisol level was lower in both Zado®-supplemented fish at three weeks, while was only lower for 1 g/kg Zado® group at six weeks sampling point (Table 3).

Table 1.

| Effect of Zado® probiotic on RBCs indices in O. niloticus at 3 and 6 weeks. |
|---------------------------------------------------------------|
| Control | 1g/kg Zado® | 2g/kg Zado® |
| 3 weeks |
| MCHC 30.5±0.0 b 31.3±0.0 a 31.4±0.0 a |
| MCH 30.5±0.0 b 30.7±0.02 a 30.6±0.02 a |
| MCV 96.2±0.0 98.4±0.02 a 97.5±0.03 a |
| PCV 25.0±0.0 b 28.7±0.02 a 28.7±0.02 a |
| Hb g/100ml 7.9±0.0 b 9.0±0.02 a 9.0±0.04 a |
| RBCs(x10/mm$^3$) 2.6±0.0 b 2.9±0.02 a 2.9±0.03 a |
| 6 weeks |
| MCHC 31.5±0.0 b 31.5±0.0 31.6±0.0 |
| MCH 30.6±0.0 b 30.9±0.0 a 30.5±0.0 a |
| MCV 97.1±0.0 b 97.9±0.0 a 96.5±0.0 a |
| PCV 27±0.0 b 29.7±0.0 a 30.0±0 a |
| Hb g/100ml 8.5±0.0 b 9.3±0.0 b 9.5±0.0 a |
| RBCs(x10/mm$^3$) 2.8±0.0 b 3.0±0.0 b 3.1±0.0 a |

Data is presented as mean ± SE. Different superscript letters indicate significance ($P < 0.05$).

3.4. Antioxidant’s activity

Gpx, catalase, and SOD significantly ($P < 0.05$) increased in response to dietary probiotic supplementation after both three and six weeks (Fig. 4 A, C, and D). The level of MDA at the end of weeks three and six was ($P < 0.05$) significantly lowered in supplemented groups than in the control group (Fig. 4 B).

3.5. Growth performance

Inclusion of Zado® probiotic revealed ($P < 0.05$) significant improvement in growth performance parameters (BW, BMG, SGR, and LGR) through the entire experimental period with the best performance for 2 g/kg Zado®-supplemented group, which also showed the most ($P < 0.05$) significant decrease in FCR compared to the control group. Somatic indices did not reveal a ($P < 0.05$) significant difference over control, except for the 1 g/kg Zado® group that showed a significant increase in both hepatosomatic and intestine somatic indexes at the sixth week compared to the start of the feeding trial (Table 4).

4. Discussion

The present study aimed to evaluate the potential effects of the addition of the commercial probiotic Zado® to the basal diet offered to Nile tilapia O. niloticus on fish hematology and immunity, liver function, stress indicators, and growth. For this purpose, the probiotic Zado® (Ruminococcus species, 28 × 10⁵ CFU/g) was mixed with the basal diet for the experimental groups at a rate of 1 and 2g/kg.

It is well noted that hematological parameters are essential to confirm the wellbeing of fish [2]. Our data showed that throughout the experimental period the average values of MCHC, MCH, MCV, and PCV and RBCs count were ($P < 0.05$) significantly higher in the Zado®-supplemented fish groups than in the control group. In the same context, WBCs values were ($P < 0.05$) significantly higher in 1 g/kg Zado® group and the percentage of monocytes and lymphocytes were significantly higher in 2 g/kg Zado® group. No significant difference was recorded in basophils percentage. Heterophils percentage was ($P < 0.05$) significantly reduced by supplementation of the probiotic. Other studies had demonstrated that hematological profiles can be modified and improved by probiotics supplementation [13,23,24]. In fact, to the best of our knowledge, information about the effects of a probiotic containing Ruminococcus sp. on hematology of fish including tilapia is scarce, thus we will discuss the effects of probiotics in general. White blood cells are considered as an essential component acting as a core to both the innate and adaptive immune response and consequently, a higher abundance implies activation of immune system [28]. Eissa and Abou-ElGheit [36] observed a higher abundance of circulating white blood cells in probiotic-fed tilapia when compared to those fed non-supplemented diets after supplementation with Ps. flescens. Those activities could be attributed to the overall improvement of immune response [30].

Regarding immune response, probiotics could enhance immune response when administered for fish orally or in water [26]. Our results showed that fish fed with Zado® supplement expressed ($P < 0.05$) statistically significant increase in the number of WBCs indices, with the highest significant increase for 2 g/kg Zado® group when compared to those fed non-supplemented diets (Table 2).

Table 2.

| Effect of Zado® probiotic on WBCs indices in O. niloticus at 3 and 6 weeks. |
|-------------------|----------------|----------------|----------------|----------------|
| Zado® g/kg | WBCs(x10³/mm³) | Lymphocytes (%) | Heterophils (%) | Monocytes (%) |
| 3 weeks |
| Control 21.1±0.0 b | 76.3±0.0 a | 14.7±0.02 a | 7.0±0.0 b | 0.7±0.02 |
| 1 30.6±0.02 a | 78.7±0.0 b | 12.0±0.0 b | 7.7±0.0 a | 0.7±0.02 |
| 2 29.8±0.0 a | 79.3±0.0 a | 11.0±0.0 b | 8.3±0.0 a | 0.7±0.02 |
| 6 weeks |
| Control 20.8±0.0 b | 75.0±0.0 a | 14±0.02 a | 7.0±0.0 b | 1±0.02 |
| 1 32.2±0.02 a | 78.0±0.0 b | 13±0.0 a | 8.0±0.0 b | 1±0.02 |
| 2 31.2±0.0 a | 79.0±0.0 a | 12±0.0 b | 9.0±0.0 a | 1±0.02 |

Data is presented as mean ± SE. Different superscript letters indicate significance ($P < 0.05$).
significantly higher values of the phagocytic index and phagocytic activity; NO activity; and lysozyme activity at three and six weeks. This may be due to the expected immunostimulation by probiotic. Similar results were reported by Telli et al. [31] and Pirarat et al. [20] who reported that dietary inclusion of *Lactobacillus rhamnosus* caused elevated serum complement activity and improved phagocytosis capability of head kidney leukocytes.

Biochemical parameters are valued biomarkers in evaluating the health status of different fish species [15]. In the current study, Throughout the entire experiment serum globulin, albumin and total protein; and AST and ALT results showed a ($P < 0.05$) significant improvement in Zado® supplemented fish. Serum glucose concentration was significantly higher in both Zado® supplemented fish at three weeks, while was only higher for 2 g/kg Zado® group at six weeks. Also, cortisol level was lower in both Zado® supplemented fish at three weeks, while was only lower for 1 g/kg Zado® group at six weeks. Similar effects were observed in two other studies [24, 30], which studied the effect of single and multi-strain probiotics, and *Enterococcus faecium* incorporation on Rohu, *Labeo rohita*, and Nile tilapia, respectively. The increased values of serum protein in supplemented tilapia might be due to the improved feed efficiency and utilization as reported

![Fig. 1. Phagocytic index and activity of O. niloticus supplemented with Zado® probiotic at 3 and 6 weeks, n = 9 ± SEM. Different superscript letters indicate ($P < 0.05$) significance difference.](image1)

![Fig. 2. Nitric oxide (NO) of O. niloticus supplemented with Zado® probiotic at 3 and 6 weeks. Values (n = 9) ± SEM with different superscript letters are ($P < 0.05$) significantly different.](image2)

![Fig. 3. Effect of Zado® probiotic on lysozyme activity in O. niloticus at 3 and 6 weeks. Values (n = 9) ± SEM with different superscript letters indicate ($P < 0.05$) significance.](image3)
previously [19,30]. Nevertheless, a high level of supplementation of the probiotic was associated with a significant increase in cortisol levels. Telli et al., [31] reported that the levels of glucose and cortisol have not been changed in response to probiotic supplementation in tilapia. Meanwhile, Iwashita et al., [13] observed increased serum glucose and cortisol concentrations in *O. niloticus*. On the other hand, 

Data is presented as mean ± SE. Different superscript letters indicate significance (*P* < 0.05).

### Table 3.
Effect of Zado® probiotic on serum protein, ALT, AST, glucose, and cortisol concentrations in *O. niloticus* at 3 and 6 weeks.

| Zado® g/kg | Globulin (g/dl) | Albumin (g/dl) | Total prot.(g/dl) | AST (U/l) | ALT (U/l) | Glucose (mg/dl) | Cortisol (ng/ml) |
|-----------|----------------|----------------|------------------|-----------|-----------|----------------|-----------------|
| 3 weeks   |                |                |                  |           |           |                |                 |
| Control   | 1.6±0.0        | 1.3±0.0        | 3.0±0.0          | 21.0±0.0  | 29.8±0.0  | 9.1±0.0        | 40.2±0.0        |
| 1         | 1.8±0.0        | 1.4±0.0        | 3.2±0.0          | 20.0±0.0  | 29.9±0.0  | 12.0±0.0       | 40.0±0.0        |
| 2         | 1.8±0.0        | 1.4±0.0        | 3.2±0.0          | 20.0±0.0  | 29.8±0.0  | 12.1±0.0       | 40.0±0.0        |
| 6 weeks   |                |                |                  |           |           |                |                 |
| Control   | 1.7±0.0        | 1.3±0.0        | 3.1±0.0          | 20.8±0.0  | 29.8±0.0  | 9.3±0.0        | 38.9±0.0        |
| 1         | 2±0.0          | 1.5±0.0        | 3.5±0.0          | 20.0±0.0  | 29.8±0.0  | 12.2±0.0       | 38.5±0.0        |
| 2         | 2±0.0          | 1.5±0.0        | 3.5±0.0          | 20.1±0.0  | 29.8±0.0  | 12.3±0.0       | 39.1±0.0        |

### Table 4.
Effect of Zado® probiotic on growth performance in *O. niloticus* at 3 and 6 weeks.

| ISI | SSI | HIS | FCR | LGR (%) | SGR (%) | BMG (%) | Final Wt (g) | Initial Wt (g) | Zado® g/kg feed |
|-----|-----|-----|-----|---------|---------|---------|--------------|----------------|----------------|
| 3 weeks |     |     |     |         |         |         |              |                |                |
| 4.20±0.0 | 0.11±0.02 | 2.83±0.0 | 2.1±0.0 | 42.3±0.0 | 1.8±0.0 | 128±0.0 | 36.5±0.5 | 16.0±0.5 | Control         |
| 4.92±0.0 | 0.10±0.02 | 2.71±0.02 | 1.9±0.0 | 45.6±0.0 | 2.0±0.0 | 145.6±0.0 | 39.3±0.7* | 16.0±0.5 | 1.0             |
| 5.13±0.02* | 0.11±0.05 | 2.63±0.0 | 1.7±0.0* | 54.4±0.0* | 2.1±0.0* | 163.8±0.7* | 42.2±0.5 | 16.0±0.5 | 2.0             |
| 6 weeks   |     |     |     |         |         |         |              |                |                |
| 3.73±0.02 | 0.11±0.02 | 2.84±0.05 | 1.0±0.03 | 65.1±0.02 | 1.11±0.0 | 215.9±0.7 | 70.5±0.5 | 16.0±0.5 | Control         |
| 4.93±0.05 | 0.10±0.00 | 3.01±0.02 | 1.2±0.02 | 72.1±0.05 | 1.14±0.0 | 226.4±0.5 | 52.2±0.5 | 16.0±0.5 | 1.0             |
| 5.03±0.0* | 0.12±0.05 | 3.13±0.0* | 1.1±0.05* | 81.7±0.02* | 1.18±0.0 | 241.1±0.5* | 54.6±0.7* | 16.0±0.5 | 2.0             |

Values are mean (n = 30) ± SEM. Asterisk (*) are different significantly (*P* < 0.05).

**ISI** = Hepatosomatic index, **SSI** = Spleen somatic index and **HSI** = Intestine somatic index.

**BMG** = Body Mass Gain, **SGR** = Specific growth rate, **FCR** = Feed Conversion Ratio.

Fig. 4. Effect of Zado® probiotic on antioxidants activity in *O. niloticus* at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Different superscript letters are different significantly (*P* < 0.05).

Fig. 4. Effect of Zado® probiotic on antioxidants activity in *O. niloticus* at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Different superscript letters are different significantly (*P* < 0.05).
increased the SGR of European Sea Bass (which also showed the most (experimental period with the best performance for 2 g/kg Zado supplementation revealed (the inclusion of probiotics in fish diets [26]. In the current study, Zado SOD levels.

Antioxidant defense capability is very important to confirm the fish’s status [10]. Levels of antioxidant enzymes as Gpx, SOD, and CAT were (P < 0.05) significantly greater in fish receiving the probiotic supplement, while pro-oxidant MDA decreased concurrently. These findings are similar to results from [21]. In a similar context, Zhou et al. [35] found an increased serum SOD in response to supplemental probiotic feeding of tilapia using containing either B. coagulans or B. subtilis. However, Ridha and Azad, [22] observed that supplemental feeding of O. niloticus using a probiotic which contained B. subtilis, B. licheniformis and S. cerevisiae for 84 days did not alter levels of SOD. Likely, the differences in the probiotic composition together with differences in the experimental period led to the observed variations in SOD levels.

Growth performance parameters are expected to be modulated by the inclusion of probiotics in fish diets [26]. In the current study, Zado® supplementation revealed (P < 0.05) significant improvement in growth performance parameters (BW, BMG, SGR, and LGR) through the experimental period with the best performance for 2 g/kg Zado® group, which also showed the most (P < 0.05) significant decrease in FCR compared to the control group. Likewise, Hasan and Banerjee [8]; Torrecillas et al. [33] demonstrated that dietary probiotics significantly increased the SGR of European Sea Bass (Dicentrarchus labrax). This improvement is a result of the fact that the probiotic has positive effects on fish growth by directly affecting absorption and appetite. This positive effects could be because of better utilization of nutrients after probiotics administration in the fish diet [3,23,26].

In conclusion, the current investigation revealed the positive role of Zado® probiotic dietary incorporation in O. niloticus diets that could be used as a safe alternative for improving hematologic, immune, antioxidant, and growth profiles of supplemented Nile tilapia.

Declaration of Competing Interest

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

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