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Immunological, Biochemical and Growth Performance Studies on Nile Tilapia Supplemented with Probiotic, Green Tea and Clove Oil

Ahmed M. Ammar[1*], Sarah Y. Abd El-Galil[1], Bassant E. Mohamed[1], Amany A. Gharib[2]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Egypt.
[2]Department of Hatchery and Fish Physiology, Central Laboratory for Aquaculture Research (CLAR), Abasa, Abo-Hammed, Sharkia, Egypt.

***Correspondence**
Ahmed M. Ammar
Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Egypt.
E-mail: Prof.ahmedammar_2000@yahoo.com

Abstract

The goal of this study was to determine how probiotic "probax®," green tea, and clove oil supplementation affected Nile tilapia (*Oreochromis niloticus*) growth performance and immune state. In a randomized full block design, 120 Nile tilapia in total, average body weights of (25.57 ± 0.3g) were randomly assigned to 4 treatments with 3 replicates (10 per replicate). Dietary treatments included either a base diet without any supplements (group 1) as the control group or a base diet supplemented with probax (group 2), green tea (group 3), or clove oil (group 4). The results showed that the groups treated with green tea and probax experienced significantly greater weight gains than the groups treated with clove oil. While specific immunological measures like IgM and CRP show a large increase in groups treated with green tea, rather than clove oil, nonspecific immune data showed that the overall leukocytic count of the groups did not differ from one another. lysozymes were higher in the probiotics-treated group, although in comparison to other treated groups, the control group had higher levels of IgG. However, globulin levels were noticeably greater in the individuals receiving probax treatment compared to other groups, whereas albumin was noticeably greater in the groups that received treatment with green tea and clove oil. As a result, the groups treated with probax and clove oil had considerably greater albumin to globulin ratios than the green tea-treated group. As a result of this study, it is possible to recommend adding probax, green tea, and clove oil to the diets of Nile tilapia as growth promoters and immunostimulants. According to this research study, green tea is the most potent of the group before being exposed to *Aeromonas hydrophila*, and it gets even stronger thereafter.

KEYWORDS
Aeromonas species, Nile Tilapia, Probiotic, Green tea, Clove oil

INTRODUCTION

Fish and their merchandise are a crucial supply of digested and scrumptious animal protein with excessive organic value (Faber *et al*., 2010). They include significant amounts of trace elements as well as numerous necessary minerals, polysaturated fatty acids, omega-3, critical vitamins, and amino acids (Abdullahi *et al*., 2001; Surette, 2008). The most significant and valuable fish species in Egypt is the Nile tilapia (*Oreochromis niloticus*), which accounts for 71.38% of all fish grown in Africa and 1.54% of all fish raised globally (FAO, 2012). After China and Indonesia, Egypt is the third-largest producer of tilapia. The most significant governorates in Egypt are Kafr-Elsheikh, Behera, and Sharkia, which provide 80% of the nation's farmed tilapia (El-Sayed, 2013). Due to the huge number of fish deaths, notably in China and India, *Aeromonas* infection in fish generates problems for the global economy (Citarasu *et al*., 2011). The pervasive handling of antibiotics in the treatment strategies upsetting an extensive distribution of resistant bacteria especially in the aquatic environment (Young, 1993). Enhancing the performance, antioxidant activity, and innate immunity of many fish species by making the best use of phytogenic natural compounds, which are frequently employed in aquafeeds, is currently improving the aquaculture business (Dawood *et al*., 2018; Dawood *et al*., 2020; Alagawany *et al*., 2021).

The use of medicinal plants in aquaculture is a novel strategy. Because of the potential for the generation of microorganisms resistant to antibiotics, environmental pollution, and the build-up of residues in fish tissue, the appropriate use of antibiotics and other chemotherapeutics in fish culture has been criticized (Ringo *et al*., 2010). Farmers typically seek out innovative, feasible alternative feed sources that improve fish development while also lowering feed prices, even though running expenses can vary (Kari *et al*., 2022). *Camellia sinensis L.*'s green tea is made from non-oxidized, unfermented leaves that contain a variety of ingredients with growth-promoting, anti-inflammatory, antioxidant, antibacterial, antiviral, antiparasitic, and immunostimulant qualities (Crespy and Williamson, 2004). The use of probiotic bacteria to improve growth performance has recently attracted a lot of attention in aquaculture. Probiotic use in aquaculture has been shown to have positive outcomes (Balcázar *et al*., 2006). Clove oil has cytotoxic, anaesthetic, antibacterial, anti-inflammatory, and antioxidant effects (Chaieb *et al*., 2007; Gülçin *et al*., 2012; Soni and Dahiya, 2014). In fish, clove oil could promote the growth of

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Nile tilapia when introduced to diets (Gaber, 2000).

Fish in rivers, and saline water are the principal reservoirs for *Aeromonas*, however Separating this pathogen from chlorinated water, including water supplies, has also proven possible. *Aeromonas* species have been added to the contamination candidate list of emerging water-borne diseases by the American Environmental Protection Agency (Borchardt *et al*., 2003). It is caused by *Aeromonas*' capacity for growth and the creation of biofilm in chlorinated water distribution systems. A Foods sold in stores, such as fish, seafood, raw milk, chicken, and red meats, have been found to be hydrophilic (Sreeremya, 2017; Tahoun *et al*., 2018; Wamala *et al*., 2018). Aerolysin, hemolysin, proteases, and lipases are a few possible virulence factors that have been linked to the pathogenicity of Aeromonads. These poisons significantly contribute to the development of illnesses in both people and fish (Umesha *et al*., 2011). Based entirely on immunological research, the hemolysins produced utilizing *A. hydrophila* are split into two main groups: extracellular hemolysin and aerolysin (Kozaki *et al*., 1989). Intake of infected fish, sea foods, and drinking water, as well as direct contact with recreational waters, are the two main ways that the zoonotic illness of aeromoniasis spreads (Yogananth *et al*., 2009). This zoonotic pathogen is a facultatively anaerobic, motile, Gram-negative, no spore-forming member of the Aeromonad family (Abbott *et al*., 2003; Martin-Carnahan *et al*., 2005). The most dangerous zoonotic pathogen is thought to be *Aeromonas*. Meningitis, fulminating septicemia, skin and wound infections, gastroenteritis, septic arthritis, and traveler's diarrhoea are among conditions that can affect people (Salunke *et al*., 2015). The flora of fish, amphibians, and reptiles includes *Aeromonas*, which is frequently found in aquatic habitats (Austin and Allen-Austin, 1985). The purpose of the study was to determine how the probiotic "probax®," green tea, and clove oil supplements impacted the growth performance and immune condition of Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Experimental fish

Healthy Nile tilapia fingerlings total number (n.=120) were obtained a live from Abbasa fish hatchery, Sharkia, Egypt. The fish displayed no clinical abnormalities and had no history of disease outbreak. Before the experiment began, fish (45.0±0.5 g) were randomly placed for two weeks into glass aquariums (10 fish/aquarium) that were pressurised air was delivered via air stones. While being given a basal diet. This study was conducted according to the guidelines of the ethical committee for the animal use of Zagazig University, Egypt.

Supplements

After the adaptation period, four groups which (G1: basal diet, G2: Probax, G3: Green tea, G4: Clove oil) of fish were created and fed one of the tested diets twice per day at 9:00am and 2:00 pm for 12 weeks until apparent satiation was reached. The tested diets represent 0.3% of body wt., consist of 0.3% Probax probiotic each kg contain $1.0X10^{11}$ CFU Bacillus subtitles and $1.0X10^{10}$ CFU Lactobacillus casei and up to 1 kg dextrose, green tea (1.5% gun powder 100% natural), and clove oil 2.5% (Table 2)

Experimental fish design

This experiment was carried out for three months (from October 2020 to January 2021) during the season at the Central Laboratory for Aquaculture Research (CLAR), Abbasa, Sharkia, Egypt.

Determining performance metrics for growth and survival

At the start of the trial (12 weeks in) and at the end (12 weeks later), the weight of the fish in each aquarium was obtained. The survival rate (SR) and growth characteristics have been recorded in line with Jayant *et al.* (2018) as follows:

Weight gain (g) = Final body weight (g) minus Initial body weight (g)

The percentage of increase in weight was computed according to Jauncey and Ross (1982) as follows:

Weight gain percent is calculated as Final average body weight - Initial average body weight/Initial average body weight multiplied by 100.

Specific growth rate (SGR) turned into decided according to Sveier *et al.* (2000): Specific growth rate (SGR) = 100 (ln W2 – ln W1) / T, Where W1 and W2 are the preliminary and final fish weight, respectively, and T changed into the range of days within-inside the feeding period.

The average body weight of fish for each group was determined after one and two months of the feeding experiment and was measured in accordance with Windell *et al.* (1978) and Siddiqui *et al.* (1988) as: Fish total weight divided by the number of fish in each group equals average body weight.

Evaluation of feed utilization

By dividing the total weight of the feed provided during a specific period by the number of fish that survived, the amount of food consumed was calculated.

Feed conversion ratio (FCR) (Sveier *et al*., 2000) was determined using the following by dividing the live weight increase in grams/dry feed consumption in grams.

Survival rate (%) was calculated as: Number of fish counted/Number of supplied fish x 100.

Blood and serum samples

Whole blood samples were collected in tubes containing EDTA as anticoagulant (blood samples from one fish from each replication, 3 fish/group) were used for estimation of total leucocyte count. Another blood samples were collected on plain tubes and processed for separation of serum that used for immunolog-

Table 1. Experimental fish design.

No. of Groups	Group 1	Group 2	Group 3	Group 4
Type of Diet	Control Diet	Probax Probiotic -0.30%	Green Tea -1.50%	Clove Oil -2.50%
No. of fishes	10	10	10	10
No. of replicates	3	3	3	3
Total No. of fish in each group	30	30	30	30

ical analysis.

Evaluation of immunological parameters

Determination of immunoglobulin M (IgM)

The immunological complex produced by IgM present in the blood sample when combined with a specific antiserum to the nephelometry technique was used to measure the quantity of IgM by measuring how these complexes scattered a beam of light passing through the sample.

Serum lysozyme activity assay

Briefly, 0.03% lyophilized in (0.05 mM) solution phosphate buffer (pH 6.2) was used as substrate. Serum lysozyme activity was assessed using the technique of Ellis (1990).

Determination of C-reactive protein

Turbidimetric Immunoassay (TIA), which employs polyclonal antibodies produced in response to liquid phase immunoprecipitation following rabbit immunization with human CRP, was used to measure the amounts of CRP in the serum (Protiline, Bio-Merieux). The development of turbidity, which increases at 340 nm and is inversely related to the amount of CRP inside the sample, is used to monitor the creation of insoluble antigen-antibody complexes (Igor and Stanislav, 2000).

Serum biochemical assays

The serum overall protein and albumin have been decided spectrophotometrically (Doumas *et al*., 1981), The amount of globulin was calculated by deducting serum albumin from the total protein.

Evaluation of Nonspecific immunological parameters (Leucocytes counts)

Total leukocytic count was performed using the improved Neubaur chamber, Natt and Herrick's solution as diluting fluid and 1:100 diluted blood according to the method described by Stoskopf *et al*. (1993). Nitroblue tetrazolium activity (NBT) was performed according to Studnicka *et al*. (1985). The Lysozyme concentrations in samples were determined from a plotted standard curve against the corresponding clear zone ring diameter on the linear axis (Rao *et al*., 2007).

Challenge study

After ninety days of feeding, 10 fishes from every remedy which include positive control had been injected with 0.1 ml of *A. hydrophila* (3.16×10^7CFU/ml) and fed with basal diet. Fishes that belonged to the negative control group were injected with 0.1 ml of phosphate buffered saline. The survival rate was recorded daily for 10 days.

Statistical model and analysis procedure

Excel was used to alter the data (Microsoft Corporation, Redmond, WA, USA) Data were subjected to analysis of variance using the statistical analysis system's general linear model process (GLM). (PROC GLM; SAS Institute Inc., 2012). The following statistical model was applied for analysis of all measurements:

$Y_{ij} = \mu + TRT_i + e_{ij}$.

Where, Y_{ij} = Observations, μ = Overall mean, TRT = effect of ith antioxidant material (i, 1 to 4), e_{ij} = random error. According to orthogonal comparisons, the differences between the control and antioxidants were looked into. Tukey's studentized range (HSD) test was used to distinguish the differences between treatment means. Shapiro-Wilk test was conducted in order to check for normality (Razali and Wah, 2011). The statistical significance was accepted at ($p<0.05$).

The mean and SEM of the results were given (Standard Error of Mean). ANOVA in one direction, then Tukey's Honestly Significant Difference (HSD) test as a post hoc analysis, and two-way repeated measures, followed by Both a post hoc test and Duncan's multiple range test were used to assess how the five treatment groups affected the various biochemical parameters. Statistical significance was indicated by the value of $P< 0.05$. Statistical Package for Social Sciences, version 24.0 (SPSS, IBM Corp., Armonk, NY) and Graph Pad Prism 8.0.2 were used for all analyses

Table 2. Composition of the basal diet used in the experiment.

Ingredients	Basic diet (G1)	Probax (G2)	Green tea (G3)	Clove Oil (G4)
Yellow Corn	34	34	33	32
Wheat flour	7	6.7	6.5	6.5
Poultry by-product meal	14	14	14	14
Soya oil	3	3	3	3
SBM local 44%	37.3	37.3	37.3	37.3
Calcium Carbonate (Caco3)	1.5	1.5	1.5	1.5
dicalcium ph.	0.2	0.2	0.2	0.2
Salts (Nacl)	1	1	1	1
Vit. & Min. premix	2	2	2	2
Probax	0	0.3	0	0
Green Tea	0	0	1.5	0
Clove Oil	0	0	0	2.5
Total	100	100	100	100
Calculated Analysis				
CP%	*28.48*	*28.44*	*28.34*	*28.25*

and visualization (GraphPad Software, Inc).

RESULTS

Results of the impact of dietary supplementation of probiotic, green tea, and clove oil on growth attributes of *O. niloticus* are presented in Table 3. Supplementing the *O. niloticus* diets with 1.5% green tea and 0.5% probiotic produced substantial variations ($p < 0.05$) in the control fish in terms of final live body weight (FBW), cumulative body weight gain, specific growth rate, feed consumption, and feed conversion ratio. Meanwhile, non- significant differences were observed between clove oil 2.5% group and the control group in feed consumption ($p > 0.05$). Similarly, non-significance differences were detected in all growth attributes between the groups treated by green tea (1.5%) or clove oil (2.5%).

Table 4 shows the two-way repeated measures ANOVA that was run to test differences in parameters that recorded in different times of experiment, before and after treatment respectively, when applying different treatments with either control diet, probiotic, green tea, clove oil. The findings showed that the interaction between the two factors (the time of experiment and type of treatment) had a very big impact.

Result revealed that the group of probiotic diet showed a highly significant increase in IgM, CRP, globulin levels after the challenge test, but showed slightly significant increases in IgM, CRP, albumin, globulin levels before challenge test.

While green tea diet resulted in highly significant increases in IgM, CRP, and globulin levels, with moderate increase in albumin level. On the other hand, green tea diet decreased IgG and A/G ratio before challenge test, However, after challenge, albumin and albumin globulin ratio were significantly increased, with a slight increase in IgM and CRP levels, and decreases in IgG and globulin levels.

Before the challenge test, clove oil diet resulted in a highly significant increase in albumin level, with slight increases in IgM, CRP, and globulin levels, and decreases of IgG and A/G ratio. Meanwhile after challenge test, there were moderate increases

Table 3. Effect of extra virgin probiotic, green tea, and clove oil supplementations on growth attributes of *O. niloticus*

Parameters	Control	Natural antioxidant treatments			*p*-value¶		
		Pro. 0.5%	GT 1.5%	Clov. (2.5%)	Treatments	Normality	Control vs. Treatments
Initial No. fish	10	10	10	10	--	--	--
Final No. fish	8	10	10	10	--	--	--
Survival rate (%)	80	100	100	100	--	--	--
Initial body weight	23.66±0.80	23.76±0.72	23.52±0.88	23.16±0.90	0.263	0.092	0.338
Final body weight	43.89±1.19	50.14±1.07‡	51.91±1.15‡	45.92±1.81+*	0.048	0.125	0.033
Body gain	20.23±0.96	26.38±0.77‡	28.39±0.91‡	22.76±0.33‡*+	0.035	0.072	0.029
Body gain (%)	85.50±2.93	111.02±4.74‡	120.71±4.89‡	98.27±3.64‡*+	0.018	0.352	0.001
Specific growth rate	0.298±0.00	0.360±0.00‡	0.382±0.00‡	0.330±0.00‡*+	0.016	0.441	0.008
Feed consumption (fish/90 days)	91.19±1.27	99.76±1.09‡	101.83±2.84‡	93.25±3.58+*	0.004	0.254	0.001
Feed conversion ratio	4.51±0.01	3.78±0.01‡	3.59±0.01‡	4.08±0.01‡*	0.044	0.422	0.034

‡ Different with the control ($p < 0.05$); * differ with green tea ($p < 0.05$); +differ with probiotic ($p < 0.05$).

Table 4. Effect on parameters before and after challenge test with *Aeromonas hydrophila*.

		G1	G2	G3	G4
IgM (IU/L)	Before challenge	399.67±32.00^{c}	546.67±13.69^{b}	660.33±29.04^{a}	506.67±43.39^{b}
	After challenge	587.00±2.65^{ab}	646.00±3.2^{a}	594.33±41.37^{ab}	502.67±25.21^{b}
lysozymes (mg/dl)	Before challenge	3.05±1.13	3.63±0.62	2.31±0.22	2.64±0.36
	After challenge	13.55±2.33	10.35±0.72	12.91±0.16	12.85±0.16
CRP (g/L)	Before challenge	25.64±5.27^{d}	55.65±8.83^{abc}	76.80±5.95^{a}	47.61±11.74^{bc}
	After challenge	62.28±2.53^{abc}	70.61±1.35^{a}	67.67±6.10^{ab}	43.96±6.13^{cd}
IgG (mg/dl)	Before challenge	353.00±32.87^{c}	211.00±1.73^{d}	203.00±5.29^{d}	216.00±38.42^{d}
	After challenge	944.33±49.87^{a}	708.67±78.28^{b}	256.33±21.87^{cd}	134.00±34.09^{d}
Total Protein (g/dl)	Before challenge	4.11±0.14	4.03±0,07	4.08±0.11	4.25±0.36
	After challenge	3.63±0.13	3.35±0.17	3.08±0.06	3.20±0.39
Albumin (g/dl)	Before challenge	2.21±0.02^{abc}	2.07±0.03^{bcd}	2.34±0.14^{ab}	2.46±0.23^{a}
	After challenge	1.78±0.04^{d}	2.03±0.10^{bcd}	2.31±0.07^{ab}	1.94±0.02^{cd}
Globulin (g/dl)	Before challenge	2.07±0.03^{a}	1.90±0.02^{ab}	2.05±0.05^{a}	1.82±0.08^{ab}
	After challenge	1.68±0.02^{b}	1.86±0.21^{ab}	0.83±0.03^{c}	0.90±0.01^{c}
A/G	Before challenge	1.07±0.01^{d}	1.09±0.03^{d}	1.14±0.08^{d}	1.35±0.07^{c}
	After challenge	1.06±0.03^{d}	1.12±0.14^{d}	2.78±0.06^{a}	2.15±0.04^{b}
WBCs (x 10^{3})	Before challenge	47.60±2.80	46.37±7.35	59.20±6.65	52.77±5.06
	After challenge	37.53±4.47	40.30±10.86	40.90±12.56	38.57±7.99

G1: Control diet; G2: Probax; G3: Green tea; G4: Clove oil groups; IgM: Immunoglobulin M; CRP: C- reactive protein; IgG: Immunoglobulin G; A/G: Albumin/globulin ratio; WBCs: White Blood Cells.
Data are presented as mean±SEM. abcMeans within the same row according to Duncan's multiple range test results, carrying distinct superscripts are considerably different at $P < 0.05$.

in IgM, albumin, A/G ratio, and decreases of CRP, IgG, globulin levels.

DISCUSSION

Commercial probiotic products contain one or more bacteria organisms as Bacillus, Streptococcus, Lactobacillus, or commercial yeast. Feed supplemented with multiple species of probiotics likely provides a wider variety of antibacterial components than mono-species probiotics (Nayak, 2010).

The obtained results showed that probiotic (0.5%) induced significant increases ($P<0.05$) in final live weight, cumulative body weight gain, specific growth rate, feed intake, and feed conversion ratio in comparison to the control group, these results agree with Geovany *et al*. (2007) who mentioned that feeding probiotics may enhance appetite and growth performance of the cultured fish species. However, the specific function of probiotics may vary according to the host animal and the probiotic characteristics. The results also agree with Ghosh *et al*. (2008) and Merrifield *et al*. (2010), who confirmed in their experiment that dietary probiotics may aid in enhancing the fish growth. The enhanced growth rate of fish is one of the many advantages of probiotics in aquaculture; this advantage is thought to result from the bacterial species colonizing the host's gut because probiotics produce a change in the gut's bacterial population that somehow improves the host's health (Balcázar *et al*., 2006; El-Haroun *et al*., 2006).

The results of the present investigation revealed non-significant differences in the feed consumption between the clove oil 2.5% group and the control group ($P>0.05$). As shown in Table 3, this result is consistent with Gaber (2000) findings that cloves are an excellent source of antioxidants that can halt the oxidative rancidity and lipid peroxidation processes. In contrast, all these positive impacts could promote growth and enhance fish health in general (Chakraborty *et al*., 2014). Nile tilapia's growth was enhanced when they were fed turmeric powder (TP) at doses of 0.5 or 4% (Mahmoud *et al*., 2014; Sanchez *et al*., 2019), or clove oil at a dose of 0.08% (Gaber, 2000).

Results from the current study showed significant differences in final live weight, total gain in body weight, specific growth rate, amount of food consumed, and feed conversion ratio ($P<0.05$) compared to the control fish, especially before challenge with *Aeromonas hydrophila* as shown in Table 3. Diets that are more alluring or elegant cause fish to consume more feed and perform better, which impacts the growth and performance of the fish. The inclusion of nutritional green tea (GT) in fish diets may also enhance vitamin uptake and nutrient utilization, which in turn may help to explain why fish are growing and eating more feed. Additionally, GT may boost the activity of beneficial microorganisms and microbial enzymes, decrease the ability of pathogens, and/or increase the number of beneficial microbes, all of which improve feed digestibility and nutrient absorption. These results back up the predictions made by (Qiu *et al*., 2004) that including Chinese medicinal herbs (CMH) in the diets of crucian carp considerably improved the growth efficiency, intestinal and hepatopancreatic protease activity, and apparent protein and fat digestibility. According to Lin *et al*. (2006), traditional CMH may also influence digestive functions by improving vitamin and enzyme absorption. When herb levels rise, this may improve the speed at which food leaves the stomach.

An essential tool for disease prevention in aquaculture is immunomodulation (Abdel Rahman *et al*., 2018; 2019). As shown in Table 4, our results indicate a significant reduction in group 4 lysozymes (12.85± 0.16), total protein (3.20 ±0.39), and globulin (0.90 ±0.01) levels. Saurabh and Sahoo (2008) noted that the bacterial challenge test is used to confirm the immunomodulatory effect of turmeric powder (TP) and clove pub powder (CBP), either alone or in combination. True lysozymes have the ability to lyse Micrococcus lysodeikticus cells (Iwama and Nakanishi, 1987). Lysozyme present in the macrophages release nitric oxide, which is a potent bactericidal reactive oxygen species, which increases the ability of the macrophages to destroy infections (Neumann *et al*., 2001). High levels of serum proteins, particularly globulin, are thought to be a key sign of fish health and are linked to an effective fish immune response (Asadi *et al*., 2012).

As shown in Table 4, the results revealed a significant increase in IgM (646.00±3.21) and IgG (708.67±78.28) in group 2, which agrees with Secombes and Fletcher (1992) and Magnadóttir (2006) who noted that the fish's innate immune system is the first and most fundamental line of defence against invading infections. The main elements of the immune system are macrophages, monocytes, granulocytes, and humoral substances such immunoglobulins. The findings concur with those of Panigrahia *et al*. (2005), who proposed that dietary probiotics increase fish immunoglobulin concentration via increasing total immunoglobulin concentration. The increased overall immunoglobulin concentration could be explained by an improved immune response brought on by the addition of *L. acidophilus* to the probiotic groups.

Assessing the accelerated safety in immunostimulant-treated fish is crucial. In order to look at the disease resistance in GT-fed tilapia, the authors of this study used the *A. hydrophila* challenge and bactericidal activity. The outcomes showed an increase in IgM (594.33±41.37) and CRP (67.67±6.10) of group (3) in Table 4, believe that when GT levels increased, fish resistance to *A. hydrophila* contamination increased, as reported by Pan *et al*. (2003) and Farhoosh *et al*. (2007). Nutritional GT's immunostimulant mechanism is unclear, although it may be caused by one or more of the additives it contains, comprising glycosides, phenolic acids, flavanols, flavanones, and the aglycones of plant pigments. Effective herbal antioxidants are present in these additions (Farhoosh *et al*., 2007; Wu *et al*., 2007; Rusak *et al*., 2008). Antioxidants are known to protect biological components from oxidative damage (Mohan *et al*., 2006). Similar outcomes were made by Ardo *et al*. (2008).

The results indicated a slightly significant increase in total protein and globulin (1.86±0.21) in the second groups as shown in Table (4), compared with the control group that fed with the basic diet. Which could be explained by the immuno-modulatory effects of probac and amphibac diets on liver cells, which increase the anabolic potential of the hepatocytes to produce blood proteins, particularly globulin. The current findings were supported by various writers (Ortuo *et al*., 2002) and were based on those of Marzouk *et al*. (2008) and Chelladurai *et al*. (2013), who claimed that the usage of probiotics significantly increased the level of total protein in the body. It may be concluded that the use of the increase of the aforementioned variables is responsible for the rise in total protein and globulin in the treatment group. In the verification of the aforementioned results.

Results from this study recorded that white blood cell count was non-significantly increased in fish of the experimental groups in comparison with the control (37.53±4.47) as shown in Table 4, this in contrast with Panigrahi *et al*. (2005) who was a necessary outcome as a result of probiotics' immune system effects, which may show up in white blood cell density and may boost phagocytosis.

CONCLUSION

We concluded from the data that adding Probax®, green tea, and clove oil to Nile tilapia diets for three months can enhance growth performance parameters and activate both its specific and non-specific immunological parameters. Green tea is the strongest of the groups before being exposed to *Aeromonas hydrophila*, and it becomes much more effective thereafter, according to our findings.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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