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Growth and Feed Utilization of Nile Tilapia, *Oreochromis niloticus* fed Diets Containing Probiotic

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Abstract: A 12-week feeding trial was undertaken to evaluate the effect of dietary graded levels of a commercial probiotic, Biogen® (containing allicin, high unit hydrolytic enzyme, *Bacillus subtilis*spores and ginseng extract) on growth, survival, feed utilization, haematology parameters and proximate composition of Nile tilapia, *Oreochromis niloticus*t. Five isonitrogenous (300 g CP kg^{-1} dry matter, DM) and isocaloric (19.3 MJ gross energy kg^{-1} DM) diets were formulated and Biogen® was supplemented in five increased levels, 0, 1, 2, 3 and 4 g kg^{-1} to formulate the five experimental diets, D1, D2, D3, D4 and D5, respectively. Fingerlings averaging 2.23±0.05g were randomly distributed into 15 glass aquaria (160 liter) and each aquarium holding 25 fish and randomly assigned to one of three replicates of the diets and offered feed to satiation to fingerlings*O. niloticus*. The control diet exhibited lower growth and feed utilization than all Biogen® supplemented diets. The highest significant ($P<0.05$) average body weight (BW), body length (BL), weight gain (WG) and specific growth rate (SGR) were recorded for fish group fed the diet D3 (2 g Biogen® kg^{-1}basal diet) followed in descending order by those fed D4, D2, D5 and D1. Also, fish group fed D3 showed the best improvement in feed conversion ratio (FCR) and protein efficiency ratio (PER). Dietary Biogen® significantly improved fish survival, increased Hemoglobin (Hb), hematocrite (Htc), count of red blood cells (RBCs) and white blood cells (WBCs) and significantly decreased serum transaminase enzymes (alanine transaminase, ALT and aspartate transaminase, AST). Protein content in whole fish body significantly ($P<0.05$) increased while fat significantly ($P<0.05$) decreased with increasing dietary Biogen® level. The highest protein and the lowest fat content was recorded for fish group fed D5 and the opposite trend was recorded fish control diet (D1). It could be concluded that the optimum Biogen level was 2 g/kg for optimum growth performance for fingerlings Nile tilapia under this experimental conditions.

Key words: Probiotic · Biogen® · Growth · Feed utilization · Nile tilapia

INTRODUCTION

Aquaculture, whoever, is an increasingly important option in animal protein production. This activity requires high-quality feeds with high protein content, which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and for better growth. Some of the most utilized growth-promoting additives are hormone [1], antibiotics [2-5], organic acids or organic salts [6, 7], spices [8] and probiotics [9, 10]. The use of probiotics in aquaculture is increasing in the last years due to their beneficial effect on cultured species. Probiotics show their effects through: suppression of pathogen growth, immunological enhancement, stimulation of growth, improvement of feed utilization and stress tolerance, etc. [11, 12]. Biogen® product is an effective growth promoter feed additive used in diets of poultry and livestock[13]. The main ingredients of Biogen® are ellicien (the product of garlic)+Ginsieng+Bacillussubtitles+high unit hydrolytic enzymes (amylolytic, lipolyric, proteolytic and cell separating enzymes). The high unit hydrolytic enzyme

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group of Biogen® may make the starch, fat and protein of feeds to be entirely dissociated and absorbed in gastrointestinal tracts of the poultry and domestic animals [14]. Biogen® can enhance the metabolism of fish body cells, increases the palatability of feed, promotes the secretion of digestive fluids and stimulates the appetite, improve the efficiency of feed utilization. Moreover, it increases the vitality of cells by supplying oxygen to whole body, helps to excrete heavy metals, inhibits aflatoxin and motions the normal endocrine system [15], improves the general health and immune responses [16]. Also allicin enhance the blood circulation in gills resulting an increase in the ability of fish to use any little amount of dissolved oxygen. Biogen® also contain ginseng extract that needed by the fish body to maintain its physiological functions and have the ability to enhance the natural body resistance through activation of immune cells [17]. The present study was conducted to determine the optimal level of Biogen® that economically enhanced growth performance and improve feed utilization of *O. niloticus*.

MATERIALS AND METHODS

Experimental Diets: The experiment was conducted at the experimental facilities of the Fish Nutrition Lab, Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. Five isonitrogenous (300 g CP kg^{-1} dry matter, DM) and isocaloric (19.3 MJ gross energy kg^{-1} DM) diets were formulated and Biogen® was supplemented in five increased levels, 0, 1, 2, 3 and 4 g kg^{-1} to representing the five diets, D1, D2, D3, D4 and D5, respectively. Biogen® was supplied from China Way-Taiwan company, a new trade name for probiotic. Biogen® composed of Allicin (the active principle of garlic), high-unit hydrolytic enzymes (proteolyticlipolytic, amylolytic and cell separating enzymes) *Bacillus subtilis* and Ginseng extract. All dry ingredients of the fish meal, soybean meal, yellow corn and wheat bran were blended for 5 min and thoroughly mixed with soybean oil and vitamin and mineral mixture (Table 1). The ingredients were mixed well and made into dry pellets using a laboratory pellet mill (California Pellet Mill, San Francisco, CA, USA). The pellets (1-mm die) were dried for 4 h at 60°C and stored at -20°C until use.

Experimental Fish and Facilities: Nile tilapia, *Oreochromis niloticus* were obtained from Abbassa hatchery, Sharkia Governorate, Egypt. Fish were transferred in a 50-liter plastic bags filled with water and oxygen to fish Lab. Prior to the beginning of the experiment, fish were acclimatized to the experimental conditions and fed commercial diet (300 g protein kg^{-1}) twice daily to apparent satiation by hand for 15 days. After acclimatization, fish (2.23±0.05 g) were stocked into fifteen glass aquaria (160 L). Three replicate aquaria were randomly assigned to each treatment and each aquarium was stocked with 25 fish. The glass aquaria were supplied with de-chlorinated tap water and were continuously supplied with compressed air. About one-third of the water volume in each aquarium was daily replaced by new aerated fresh water after cleaning and removing of the accumulated excreta. A photoperiod of 12 h light, 12h dark (08.00 to 20.00) was used. Fluorescent ceiling lights has supplied the illumination. Fish were fed their respective diets by hand. Fish were given the diets to satiationtwo times a day (9:30 and 14.00) and the dead fish were daily removed and recorded.

Water temperature, dissolved oxygen, pH and total ammonia were monitored during the study, to maintain water quality at optimal range for Nile tilapia. Water temperature and Dissolved oxygen (DO) was recorded daily at 13.00 h using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and pH was recorded daily at 09.00 h using a pH meter (Orion pH meter, Abilene, Texas, USA). Total ammonia was measured two times a week according to APHA [18]. During the period of the experiment, the water-quality parameters were averaged (±SD): Water temperature was 26.43±0.5°C: dissolved oxygen, 6.2±0.3 mg/L: pH 8.23±0.4 and total ammonia, 0.12±0.05 mg/L. All tested water quality criteria were suitable and within the acceptable limits for rearing the Nile tilapia, *O. niloticus* fingerlings [19].

Survival and Growth Indices: Dead fish were daily removed and recorded. Also, body weight and body length were individually measured for each aquarium at the initiation and the termination of the experiment. Survival rate, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using the following equations: WG (g/fish)=*FBW–IBW*; SGR% = [*lnFBW- ln* IBW]/t×100, where FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t=time in days. FCR=*FI /WG*, where FI is feed intake (g); PER=*WG*/*protein intake* (g).

Survival rate = $(N_1-N_2)/N_1$ where N_1 and N_2 are the initial and final fish number, respectively.

Table 1: Composition and chemical analysis of the experimental diets

	Experimental diets				
Feed ingredients	D1	D2	D3	D4	D5
Fish meal (65%)	160	160	160	160	160
Yellow corn	280	280	280	280	280
Soybean meal (40%)	400	400	400	400	400
Wheat bran	105	104	103	102	101
Soybean oil	25	25	25	25	25
Vit. & Min. mixture[1]	30	30	30	30	30
Biogen®	0	1	2	3	4
Sum	1000	1000	1000	1000	1000
Chemical analysis (% dry matter basis)					
Dry matter (DM)	7.44	6.55	6.12	7.15	5.89
Crude protein (CP)	30.18	30.66	30.71	30.80	30.91
Ether extract (EE)	6.44	6.23	6.57	6.20	6.36
Crude fiber (CF)	9.93	8.22	8.10	8.24	8.66
Ash	7.12	7.14	7.33	7.45	7.15
NFE[2]	46.33	47.75	47.29	47.31	46.92
Gross energy (MJ kg^{-1} diet)[3]	19.33	19.31	19.35	19.26	19.35

[1] Vitamin & mineral mixture/kg premix: Vitamin D_3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

[2] Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)

[3] Gross energy calculated using gross calorific values of 0.2363, 0.3952 and 0.1715 MJ/g for protein, fat and carbohydrate, respectively according to Brett [59]

Hematological and Biochemical Blood Indices: At the end of the experiment, blood samples were collected from the caudal vein of all treatments and divided into two portions. The first portion was collected with anticoagulant 10% ethylenediaminetetraacetate (EDTA) to determine the hematocrite (Htc) and hemoglobin (Hb) according to the standard methods as described by Rawling *et al*. [20]. Total count of red blood cells (RBC"s) and white blood cells (WBC"s) were carried out by the method described by Martins *et al*. [21]. The second portion of the blood samples was allowed to clot overnight at 4°C and then was centrifuged at 3000 rpm for 10 min. The non-hemolysed serum was collected and stored at -20°C until use. Levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) were estimated according to the method described byReitman and Frankel [22].

Proximate Analysis of Fish and Experimental Diets: At the experiment termination, three fish were chosen at random and exposed to the proximate analysis of whole fish body according to the methods of AOAC [23]. Fish and diet samples were oven-dried 105°C for 24 h, ground and stored at -20°C for subsequent analysis. Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Ash by incineration at 550°C for 12 hour. Crude protein was determined by micro-Kjeldhal method, N×6.25 (using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden and crude fat by Soxhlet extraction with diethyl ether (40-60°C). Crude fiber content of diets was determined using the method of Van Soest *et al.* [24]. Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, crude fiber and ash then subtracting this sum from 100.

Statistical Analysis: Statistical analysis of the obtained data was analyzed according to SAS [25]. Differences between means were tested for significance according to Duncan's multiple rang test as described by Duncan [26].

RESULTS

Biological performance of fish: Initial Body weight (IBW) and initial body length (IBL) values ranged from 2.18-2.25 g for IBW and 4.95-5.15 cm for IBL with insignificant differences among the different fish groups (Table 2). The experiment showed final body weight (FBW) 9.46-12.49 g and andlength (FBL) 7.98-8.90 cm for different fish group and the differences in FBW and FBL were significant ($P<0.01$). Weight gain (WG) and specific growth rate (SGR) significantly varied from 7.23 to 10.90 g/fish and from 1.60 to 1.98% for SGR. The highest significant averages of body weight (BW), weight gain (WG) and specific growth rate (SGR) were recorded for fish fed D3 (2 g Biogen® kg^{-1}) followed in descending order by fish groups fed D4, D2, D5 and D1. Fish fed D1

Table 2: Growth performance of *O. niloticus* fed dietary increasing levels of Biogen®

	Body weight/g		Body length/cm			
Diets	Initial	Final	Initial	Final	WG	SGR
D1	2.24	9.46^{c}	5.15	7.98^{c}	7.23^{e}	1.60^{e}
D2	2.23	11.58^{b}	5.07	8.57^{ab}	9.35^{c}	1.83^{c}
D3	2.18	13.11^{a}	4.97	8.90^{a}	10.90^{a}	1.98^{a}
D4	2.25	12.49^{ab}	5.01	8.88^{a}	10.23^{b}	1.90^{b}
D5	2.24	10.39^{bc}	4.95	8.31^{bc}	8.15^{d}	1.71^{d}
Standard error	0.07	0.40	0.09	0.12	0.08	0.01
Probability	0.847	0.008	0.900	0.005	0.007	0.050

Averages within each column followed by different superscript letters are significantly different (P<0.05)

Table 3: Feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR) of *O. niloticus* fed dietary increasing levels of Biogen®

Diets	FI	FCR	PER	SR
D1	28.15^{b}	3.91^{a}	0.86^{b}	92.00^{c}
D2	32.30^{a}	3.46^{a}	0.97^{b}	95.00^{b}
D3	26.33^{b}	2.42^{b}	1.39^{a}	96.67^{b}
D4	27.49^{b}	2.71^{b}	1.24^{a}	95.00^{b}
D5	26.93^{b}	3.30^{a}	1.01^{b}	98.33^{a}
Standard error	1.05	0.16	0.02	0.55
Probability	0.004	0.004	0.002	0.002

Averages within each column followed by different superscript letters are significantly different (P<0.05)

Table 4: Blood parameters and liver functions of *O. niloticus* fed dietary increasing levels of Biogen®

Diets	Hb (g dL^{-1})	Htc(%)	RBC's (10^6)	WBC's (10^5)	ALT	AST
D1	6.99^{b}	27.48^{b}	2.13^{b}	9.14^{b}	46.12^{a}	47.84^{a}
D2	9.15^{a}	29.22^{a}	2.53^{a}	11.24^{a}	41.26^{b}	42.22^{b}
D3	9.87^{a}	29.29^{a}	2.73^{a}	11.76^{a}	42.13^{b}	42.14^{b}
D4	9.89^{a}	30.23^{a}	2.70^{a}	12.22^{a}	42.25^{b}	42.12^{b}
D5	10.03^{a}	30.12^{a}	2.82^{a}	12.13^{a}	41.32^{b}	41.50^{b}
Standard error	0.83	1.32	0.26	1.23	0.77	0.98
Probability	0.063	0.043	0.031	0.022	0.0020	0.0031

Means followed by the different superscript letters in each row for each trait are significantly different (P<0.05).

Table 5: Proximate composition of *O. niloticus* fed dietary increasing levels of Biogen®

Diets	DM	CP	Fat	Ash
D1	30.49	65.45^{c}	24.29^{a}	10.05
D2	32.21	66.39^{b}	22.53^{b}	10.26
D3	30.50	67.48^{b}	21.82^{b}	10.14
D4	31.41	69.54^{a}	20.08^{b}	10.16
D5	31.05	69.72^{a}	19.36^{b}	10.70
Standard error	0.63	2.23	1.34	0.96
Probability	0.075	0.033	0.025	0.081

Averages within each column followed by different superscript letters are significantly different (P<0.05)

showed the lowest growth performance indices (BW, BL, WG and SGR) in comparison to the different Biogen® levels (Table 2). Averages of feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) ranged between 26.93-32.30 g/fish, 2.42-3.91 and 0.86-1.39%, respectively. The high Biogen® levels 2, 3 or 4 g kg^{-1} in D3, D4 or D5 significantly (P<0.01) improved FCR and PER. Fish groups fed the basal diet supplemented with 2 or 3 g biogen kg^{-1} diet (D3 or D4) showed the best significant (P<0.01) FCR and PER. Statistical differences (P<0.05) were found in terms of FCR and PER and paralleled to that obtained for growth performance (BW, BL, WG and SGR). Survival rate (SR) of *O. niloticus* found to be 92.00, 95.00, 96.67, 95.00 and 98.33 for fish groups fed the experimental diets, D1, D2, D3, D4 and D5, respectively. All levels of Biogen® significantly improved fish survival and D5 showed the highest SR (98.35%) while fish fed control diet (D1) showed the

lowest (92.00%) SR. Hemoglobin (Hb), hematocrite (Htc), count of red blood cells (RBCs) and white blood cells (WBCs) significantly increased with increasing Biogen® levels in fish diets (Table 4). On the other hand, all Biogen® levels significantly ($P<0.01$) decreased transaminase enzymes (alanine transaminase, ALT and aspartate transaminase, AST).

Proximate Composition of Fish: Crude protein in whole fish body was significantly ($P<0.05$) increased with increasing dietary Biogen® levels and the opposite trend was observed for fat (Table 5). Fish group fed D5 showed the highest protein and the lowest fat content while control group (D1) gained the lowest protein and the highest fat content of whole body. Dry matter ranged between 30.49 and 32.21 while ash content ranged between 10.05 and 10.70%. Fish fed control diet showed the lowest dry matter (DM) and ash content compared to the other groups and the diet supplementation with the graded levels of Biogen® did not significantly altered dry matter or ash content of *O. niloticus* whole body.

DISCUSSION

Biological Performance of Fish: Recently, probiotics and prebiotics have become integral parts of aquaculture practices for improving growth performance [27-30]. Results of the present study showed that, all Biogen® levels (1 to 4 g kg^{-1}) give the best growth performance indices (BW, Bl, WG and SGR) compared to control diet (D1) and the better growth performance was observed in *O. niloticus* fed Biogen® supplemented diets with a trend towards the best results being achieved at a level of 2 g Kg^{-1} of Biogen® (D3). The present results confirms the previous findings. [16, 31, 32] Another studywith highest dietary Biogen® (3 g kg^{-1} diet) , significantly improved growth indices and survival rate in Shrimp, *Penaeusmonodon* [33].

The growth promoting effect of Biogen® could be attributed to the role of some Biogen® enzymes (hydrolytic, amylolytic, lipolytic, proteolytic and cell separating enzymes) which reached at certain level to act as a growth promoter through the increase in digestibility and absorbability of different nutrients in the fish gastrointestinal tract. *B. subtilis*one component of Biogen® thathave been shown to produce digestive enzymes as amylase, protease, lipase which may enrich the concentration of intestinal digestive enzymes or its effect in improving digestive activity by synthesis of vitamins and co-factors or enzymatic improvement [34]. Growth enhancement as a result of probiotic administration has been reported in several studies on a variety of fish species fed dietary probiotics *Bacillus* spp in African catfish *Clariasgariepinus* [12], *B. subtilis*, for *O. niloticus* [10, 26], *Bacillus amyloliquefaciens* in *O. niloticus* [35], *Enterococcus faecium* and *Bacillus coagulans*for*O. niloticus* [36] and *Bacillus*spp for Florida pompano and common snook larvae [30].

The enhancement in body weight gain as a result of other various components of Biogen® such as allicin, which is one of the garlic by-product which stimulated growth because of its thyroid like activity [37]. Lun*et al*. [38] reported that, allicin can activate and coordinate the function of various endocrine glands and thus enables them to secrete hormones especially the growth hormone, also allicin decrease the level of uric acid in fish feces resulting in the decreases in the level of ammonia in the water leading to a good water quality suitable for better growth rate. Ginseng one of Biogen® component which could have a growth promoting ability via prevention and treatment of sub-clinical infections, the same findings were observed by Galal *et al*.[39].

Survival rates (SR) observed amongst *O. niloticus* during the trial experiment were significantly ($P<0.05$) high in fish groups fed Biogen® supplemented diets in comparison with the control group (Table 3). Probiotic actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and space and alteration of the microbial metabolism. It also improves the nutrition by detoxifying the potentially harmful compounds in feeds by denaturing the potentially indigestible components in the diet by hydrolytic enzymes including amylases and proteases, by producing vitamins such as biotin and Vitamin B_{12} by producing inhibitory compounds [40] and by stimulating the immunity [41]. *B. subtilis* (contained in Biogen®) is currently being used for oral bacteriotherapy and bacterioprophylaxis of gastrointestinal disorders (mostly as a direct result of antibiotic treatment). Ingestion of significant quantities of *B. subtilis*and *B. pumilus* is thought to restore the normal microbial flora following extensive antibiotic use of illness [28]. The use of the multispecies of probiotic (*Bacillus* sp., *Pediococcus*sp., *Enterococcus* sp. and *Lactobacillus* sp.) at 1.0×10^6 CFU kg^{-1} might enhance protection against pathogen outbreak and increase nutrient absorption of Senegalese sole, Soleasenegalensis [42].

The obtained results (Table 3) indicated that feed intake (FI) was significantly affected by Biogen® compounds (garlic, ginger, *B. subtilis* and digestive

enzymes). Nile tilapia fry fed the diet containing 1 g kg^{-1}Biogen® consumed more feed than those fed the other experimental diets. Biogen® have a response as a palatability enhancer with better taste that increase the feed consumption by fish and consequently increase the growth rate and this may be due its content of different enzymes that increase the digestibility and absorbability of feed and activation of the intestinal villi.

Increasing Biogen® level in the experimental diets up to 2 g kg^{-1} (D3) significantly improved FCR and PER whereas the highest Biogen® level 4 g kg^{-1} (D5) re-increased feed required for the production of the same WG unit. The best FCR values observed with probiotic-supplemented diets suggest that addition of probiotics improved feed utilization. In practical terms, this means that, probiotic use can decrease the amount of feed necessary for animal growth, which could result in production cost reductions. The PER results indicate that supplementing diets with probiotics significantly improves dietary protein and energy utilization and contributes to an optimized protein use for the growth of tilapia. The present results revealed that, the use of the probiotic Biogen® as a feed additive for Nile tilapia is recommended to stimulate productive growth performance and nutrient utilization.

Feed utilization was the highest in Nile tilapia fed the Biogen® supplemented diets, meaning that the nutrients were more efficiently used for growth performance. Biogen® have a response as a palatability enhancer with better taste that increase the feed consumption by fish and consequently increase the growth rate and this may be due its content of different enzymes that increase the digestibility and absorbability of feed and activation of the intestinal villi. Biogen contain allicin, which promotes the performance of intestinal flora, thereby improving digestion and better utilization of energy, leading to improved growth [17].

Supplementation of *O. niloticus* diet with Biogen® showed significant ($P<0.05$) increase in hemoglobin, hematocrite, count of red blood cells (RBCs) and white blood cells (WBCs) (table 4). Hematological variables are commonly used as indicators of physiological condition in fish [48]. Some studies reported that after feeding with *Bacillus sp*., Nile tilapia presented alterations on the hematological variables [27, 36, 49] or not [11]. In the present study, we observe a significant increase in hematocrite, hemoglobin, count of red blood cells (RBC's) and white blood cells (WBC's) in fish fed Biogen® supplemented diets compared to control group. This could be related to the necessity of transport more oxygen in blood to meet the increasing energy demand of fish promoted by high levels of glucose [50]. As in current study, Reda and Selim[35] and Silva *et al.*[51] observed an increase in hemoglobin levels in blood of Nile tilapia supplemented with *B. amyloliquefaciens*. The authors also reported that probiotic bacteria enhance iron absorption due to the release of organics acids in the gut. This would increase the availability of iron to produce hemoglobin in fish [52]. Also, Mehrim[53]indicated that, mono-sex Nile tilapia *O. niloticus* fed the basal diet supplemented with 3g Biogen Kg^{-1} diet for 14 weeks significantly showed an increase in WBCs count. Aly*et al*. [54] reported that, total leucocytes cells was significantly increased in *O. niloticus* fed Bacillus spp. [36] indicated that, *O. niloticus* fed diets supplemented with *Enterococcus faecium* (107cfu g-1) and Bacillus coagulans (107cfu g^{-1}) significantly increased Hb, Htc, RBCs and WBCs.

Compared to control fish group, all dietary Biogen® levels significantly ($P<0.05$) decreased serum transferase enzymes (ALT and AST). The obtained results indicated that dietary probiotic Biogen® play a role in removing the toxic factors presented in the diets and therefore improved liver function. Garlic (one component of Biogen®) inhibits the fatty acids synthesis and other lipid components in liver and reduces the level of fat accumulation in liver leading to a decrease in liver weight [55]. Garlic contains a variety of organosulphur compounds, amino acids, vitamins and minerals [56]. Sulphur compounds of garlic are responsible for inhibition of cholesterol synthesis [57].

Proximate Composition of Fish: The efficiency of nutrient transfer from food to the organism can be measured by the proximal composition analysis. This is of great importance due to it may reflect the nutritional value of the fish and its organoleptic characteristics [58]. Dry matter and ash content showed some variations (but not significant) among the increased Biogen® levels in the diet. Biogen® significantly ($P<0.05$) increased protein and decreased fat content of the whole fish bodies (Table 5). This expected as fish in all treatments did not grow essentially at the same rate. Several authors have reported improvement (increased protein and decreased fat) in body composition of Nile tilapia supplemented with probiotics [27, 35]. On the other hand, Telli*et al.,* [49] and Silva *et al*., [51] showed no significant difference between Nile tilapia fed diet supplemented with *B. subitilis* and control groups.

CONCLUSION

From the results of this experiment, we concluded that, dietary administration of 2 g Biogen® kg^{-1} can be used as a probiotic agent in *O. niloticus* culture to enhance fish health, survival, feed efficiency and growth performance suggesting its use will be beneficial for the aquaculture industry of this species.

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