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EFFECT OF PROBIOTICS, PREBIOTICS AND THEIR COMBINATION ON GROWTH PERFORMANCE; LIVER FUNCTION AND HEMATOLOGICAL PARAMETER OF NILE TILAPIA (*Oreochromis niloticus*)

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Abstract

A 12-week feeding trial was conducted to evaluate the effect of dietary probiotic, prebiotic and their combination on growth, feed utilization, liver function and hematological parameter of Nile tilapia *O. niloticus*..Four isonitrogenous (297 g CP kg^{-1}) and isocaloric (19.7 MJ gross energy kg^{-1}) diets were formulated. The first diet (D1) without feed additive (control), the other three diets were supplemented with 15 g kg^{-1} Pro-Pac as probiotic (D2), 15 g kg^{-1} of Imo-Zin as prebiotic or a combined of 7.5 g Pro-Pac +7.5 g Imo-Zin /kg diet (D4). Fingerlings averaged 3.17±0.05g were randomly distributed into 12 fiberglass tanks (200 liter) and each tank holding 20 fish and randomly assigned to one of three replicates of the diets and offered feed to apparent satiation twice daily at 09:00 am and 3:00 pm.

After 12 weeks, fish fed the diets supplemented with probiotic (D2), probiotic (D3) or their combination (D4) showed the highest significant body weight (BW), body length (BL), weight gain (WG) specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and survival rate than those fed the control diet. In the same trend, the highest red blood cells count (RBCs), Hemoglobin (Hb), hematocrite (Htc), globulin and total protein were recorded for fish fed the diet supplemented with probiotic, prebiotic or their combination compared with control group (D1). On the other hand, fish fed the diet supplemented with probiotic, prebiotic or symbiotic recorded the lowest ($P<0.001$) serum transaminase enzymes (alanine transaminase, ALT and aspartate transaminase, AST). The highest significant

(P<0.05) protein and the lowest ether extract content of the whole fish body were recorded for fish group fed the diet supplemented with probiotic (D2) while control group showed the opposite trend. Ash content did not significantly (P>0.05) affected by the two feed additives Pro-Pac or Im-Zin in D2, D3 or D4.

Key words: Nile tilapia, probiotics, prebiotics, symbiotic.

INTRODUCTION

The use of probiotics and prebiotics has been regarded during recent years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming as an integral part of aquaculture practices for improving growth and disease resistance (Rombout *et al.,* 2010). This strategy offers innumerable advantages to overcome the limitation and side effects of antibiotics and other drugs and also leads to high production (Sahu *et al.,* 2008).

In recent years there has been a growing interest in understanding the mechanism of action of probiotics and prebiotics. Probiotics activity is mediated by a variety of effects that are dependent on the probiotic itself, the dosage employed, treatment duration and route and frequency of delivery. Some probiotics exert their beneficial effects by elaborating antibacterial molecules such as bacteriocins that directly inhibit other bacteria or viruses and, activity participating in the fight against infections; whereas, others inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules or modulate the inflammatory/immune response (Cerezuela *et al.,* 2011). On the other hand, the potential mechanism of prebiotics includes a selective increase/decrease in specific intestinal bacteria that modulate local cytokine and antibody production, an increase in the intestinal short chain fatty acids production, an enhanced binding of these fatty acids to G-coupled protein receptors on leycocytes, an interaction with carbohydrates receptors on intestinal epithelial and immune cells, and partial absorption resulting in a local and systemic contact with the immune system (Seifert and Watzl, 2007).

The alternative methods of disease prevention have been used as a means of reducing the presence of opportunistic pathogens and stimulating the host immune responses. However, other effects related have been observed, as improve growth performance, feed utilization, digestive enzyme activity, antioxidant enzyme activity, gene expression, disease resistance, larval survival and gut morphology alter the gut microbiota, mediate stress response, improve nutrition, produce lactase, alleviate symptoms of lactose intolerance and malabsorption (Rombout *et al.*, 2010, Ringø *et al.,* 2010 and Dimitroglou, *et al.*, 2011).

Synbiotic is defined as a combination of probiotic and prebiotic. It is presumed to impart the beneficial effects of both ingredients. Synbiotics can help to improve health status, disease resistance, growth performance, feed utilization, carcass composition, gastric morphology, and digestive enzyme activities. As such; many commercial dietary formulations now routinely include probiotics or prebiotics (Zhang *et al.,* 2010; Hassaan *et al.*, 2014&2017).

Therefore, the aim of the present study is to investigate the effects of supplementation of a commercial probiotic (Pro-Pac) and the prebiotic (Imo-Zin) and their symbiotic interaction (Pr0-Pac+Imo-Zin) on growth performance, chemical composition, hematological and biochemical blood parameters of the Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

Commercial feed additives:

Pro Pac is a commercial probiotic produced by Pro-Byn International, inc, USA and distributed by Nutrivet animal health, Maddy, Cairo, Egypt. Pro-Pac is a synergistic blend betaine, the lactic producing bacteria, *Lactobacillus acidophilus, L. plantarum*; *Enterococcus faecium*; *Bifidobacterium bifidum* and amylase, Beta- glucanase and hemicellulose enzymes from microbially derived soluble in a water soluble dextrose carrier. Each Kg of Pro-Pac contains 100 g/kg of *L. acidophilus* (1×10^8 CFU g^{-1}), 4.8 g/kg *L. plantarum* (4.8×10^7 CFU g^{-}

^{1}), 50 g/kg *E. faecium* (5×10^{7} CFU g^{-1}), 2 g kg^{-1} *B. bifidum* (2×10^{6} CFU g^{-1}), 50 g/kg *Aspergillus oryzae* fermentation extracts and 50 g/Kg *Bacillus subtilus* fermentation extracts.

Imo-Zin is a commercial prebiotic produced and distributed by Baytara for pharmaceutica technology Each 1 kg contains 232.5 g Iso-maltooligocaccharide, 186.0 g, Betaine, 6.958 g zinc and 50 g Sorbitol.

Experimental diets:

Four isonitrogenous (297.0 g CP kg^{-1} dry matter, DM) and isocaloric (19.7 MJ gross energy kg^{-1} DM) diets were formulated (Table 1). The first diet (D1) without additive (control), the second (D2) was supplemented with 15 g kg^{-1} diet of the commercial probiotic, Pro-Pac. The third diet (D3) was supplemented with a commercial prebiotic Imo-Zin (15 g kg^{-1} diet), while fourth diet (D4) was supplemented with 7.5g Pro-Pac + 7.5g kg^{-1} diet. All dry ingredients of the fish meal, soybean meal, yellow corn and wheat bran were blended for 5 min and thoroughly mixed with corn oil, codfish oil and vitamin & mineral mixture (Table 1). The ingredients were well mixed with the feed additives (probiotic and prebiotic) and the final mixtures were pelleted 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:

The experiment was conducted Central Laboratory (CLAR) for Aquaculture Research, Abbassa, Abou-Hammad, Sharkia, Egypt. Nile tilapia *Oreochromis niloticus* were obtained from the hatchery of CLAR. Fish were acclimated to the wet lab conditions for two weeks in a fiberglass tanks. During the acclimation period, fish were fed a control diet (29.70 % crude protein) to apparent satiation. After the acclimatization, the experimental fish were distributed randomly into twelve fiberglass tanks (200 liter for each) representing four treatments (three replicates for each treatment). Twenty fish (3.17±0.025 g) were randomly stocked into each tank. During the experiment, fish were hand-fed their respective diets to apparent satiation twice daily at 09:00 am and 3:00 pm a 6

days/week. About one-third of water in each pond was daily renewed by the outlet at the bottom of the pond before feeding. All tanks were provided with continuous aeration to maintain the dissolved oxygen level near saturation and fish were held under natural light.

Table 1. Composition and chemical analysis of the basal diet (g/kg).

Item	D1	D2	D3	D4
Fish meal	104.0	104.0	104.0	104.0
Soybean meal	449.8	449.8	449.8	449.8
Ground corn	223.2	223.2	223.2	223.2
Wheat bran	142.2	127.2	127.2	127.2
Codfish oil	28.5	28.5	28.5	28.5
Corn oil	22.3	22.3	22.3	22.3
Pro-Pac	0	15.0	0	7.5
ImoZin	0	0	15.0	7.5
[1]Vit. &Min. mixture	30.0	30.0	30.0	30.0
Proximate analysis %				
Dry matter	89.17	89.11	89.27	89.22
Crude protein	29.70	29.81	29.77	29.66
Crude fat	7.66	7.56	7.62	7.61
Ash	6.20	6.22	6.21	6.24
[2]Total carbohydrate	56.44	56.41	56.40	56.49
[3]Gross energy MJ kg^{-1}	19.73	19.74	19.72	19.70

[1]Vitamin and mineral mixture kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I),

[2]Total carbohydrate =100-(CP + EE+ Ash).

[3]Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj/g for protein, fat and carbohydrate, respectively according to Brett (1973).

Water temperature and dissolved oxygen were measured every other day using a YSI model 58 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). pH was monitored twice weekly using a pH meter (Orion pH meter, Abilene, Texas, USA) (APHA, 1992). Water temperature was 26.17±0.8 °C: dissolved oxygen, 5.6±0.8 mg L^{-1}: total ammonia, 0.18±0.12 mg $L^{-1}$and pH 8.52±0.3. Water quality criteria were suitable and within the acceptable limits for rearing the Nile tilapia *O. niloticus* fingerlings (Boyd 1990).

Growth and nutrient utilization parameters:

Growth performance and feed utilization parameters were measured using the following equations: Weight gain (WG) = final weight (g)–initial weight (g), Specific growth rate (SGR) = $\frac{InW2 - InW1}{t} x\,100$ Where: In = the natural log; W_1 = first fish weight; W_2 = the following fish weight in gram and t = period in days, Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g), Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g), Protein productive value (PPV) % = (protein gain (g)/protein intake (g) ×100).

Blood sample and hematological and biochemical analysis:

At the end of the experiment, blood samples were collected from the caudal vein of all experimental fish treatments and were divided into two groups. The first group was collected with anticoagulant 10% ethylenediaminetetraacetate (EDTA) to measure hematocrit (Htc), haemoglobin (Hb), red blood cells (RBCs) and white blood cells (WBCs). Htc was determined as described by Reitman and Frankel (1957), haemoglobin (Hb) was determined by the haemoglobin kit which is a standardized procedure of the cyanomet haemoglobin method and the total count of white blood cells (WBCs) was carried out by the indirect method (Martins *et al.,* 2004). The second group of the blood samples was allowed to clot overnight at 4°C and then 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 determined according to the method described by Reitman and Frankel (1957). Total protein (TP) and albumin were determined by the method of (Wotton and Freeman, 1982).

Proximate analysis of diet and fish:

At the initiation and termination of the trail a random sample of five individual fish were sampled from each tank, then oven-dried 105◦C for 24 h, ground, and stored at -20°C for subsequent analysis. Proximate analysis was conducted on diets and fish samples. Moisture, total lipids, crude protein and ash contents were all determined by the standard (AOAC, 1995). Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Ash by incineration at 550°C for 12 h. Crude protein was determined by micro-Kjeldhal method, N×6.25 (using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Sechelt extraction with diethyl ether (40 - 60°C). Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, ash and moisture then subtracting this sum from 100.

Statistical analysis:

All data were analyzed by SAS (1996). One-way analysis of variance (ANOVA) was used to determine whether significant variation existed between the treatments. When overall differences were found, they were tested by Duncan's multiple rang test as described by Duncan (1955). Two-way ANOVA was used for analyzing the individual effects of *B. licheniformis* and yeast extract, and the interaction between them. All differences were considered significant at ($P<0.05$).

RESULTS

Growth performance:

The growth response and feed utilization data are summarized in Table 2. After 12 weeks of treatment, supplementation of the basal diet with probiotic (Pro-Pac), prebiotic (Imo-Zin) and their combination, symbiotic (Pro-Pac+Imo-

Zin) significantly improved the final body weight (BW), body length (BL), weight gain (WG) and specific growth rate (SGR) of Nile tilapia compared with the control group. The highest weight BW (28.92g); the longest body length BL (11.39 cm); the highest weight gain WG (25.75 g) and SGR (2.61% day^{-1}) were recorded for fish group fed D4 (the diet supplemented with symbiotic, Pro-Pac+Imo-Zin) while fish fed the un-supplemented diet (D1) showed the lowest BW (19.45 g); shortest BL (8.87 cm), the lowest WG (16.28 g) and SGR (2.17% day^{-1}) and the differences among the different treatments in these indices were significant (P<0.05).

Survival rate:

Survival rate of fish group fed the control diet (D1) (Table 2) showed the lowest (87.50%) survival rate and feed supplementation with the probiotic (Pro-Pac), prebiotic (Imo-Zin) and symbiotic (Pro-Pac+Imo-Zin) significantly (P<0.05) improved survival rate of *O. niloticus* to 99.50, 96.00 and 97.00% for fish groups fed the different treatments D2, D3 and D4, respectively (Table 2).

Table 2. Effect of probiotic, prebiotic and their combination on growth performance and feed utilization of Nile tilapia *O. niloticus*.

	D1	D2	D3	D4	SE	*P value*
Final body weight (g)	19.45 ^{c}	23.37 ^{b}	23.92 ^{b}	28.92 ^{a}	0.38	0.001
Final body length (cm)	8.87 ^{c}	11.73 ^{a}	11.27 ^{b}	11.39 ^{b}	0.36	0.011
Weight gain (g/fish)	16.22 ^{c}	20.75 ^{b}	20.21 ^{b}	25.76 ^{a}	0.64	0.021
Specific growth rate (% day^{-1})	2.17 ^{c}	2.38 ^{b}	2.40 ^{b}	2.61 ^{a}	0.26	0.001
Survival rate (%)	87.50 ^{b}	99.50 ^{a}	96.00 ^{a}	97.50 ^{a}	0.04	0.032
Feed intake g fish^{-1}	31.51 ^{d}	39.56 ^{a}	34.21 ^{c}	36.18 ^{b}	0.53	0.021
Feed conversion ratio (FCR)	1.94 ^{a}	1.91 ^{a}	1.69 ^{b}	1.40 ^{c}	0.24	0.001
Protein efficiency ratio (PER)	1.73 ^{c}	1.76 ^{c}	1.99 ^{b}	2.40 ^{a}	0.06	0.021
Protein productive value (PPV)	42.91 ^{c}	43.70 ^{c}	49.23 ^{b}	59.35 ^{a}	0.41	0.001

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

Feed utilization:

Supplementation of tilapia diets with probiotic (D2), prebiotic (D3) or symbiotic (D4) (Table 2) significantly (P<0.05) increased feed intake improved

feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) and fish group fed D4 showed the highest feed intake (g/fish), the best feed conversion ratio (FCR), the highest protein efficiency ratio (PER) and protein productive value (PPV) compared to the control and the other treated fish groups.

Hematological and liver function indices:

Supplementation of the basal diet with probiotic, prebiotic or symbiotic significantly increased red blood cell counts RBCs ($P<0.001$), hemoglobin (Hb) level ($P<0.05$) (Table 3) and hematocrite, Htc ($P<0.001$) and fish group fed D4 showed the highest RBCs, Hb and Htc compared to untreated (D1) and the other treated fish groups (D2 and D3). Albumin, globulin and total protein ranged between 1.38 to 1.68; 0.93 to 1.67 and 2.61 to 3.24 g/dl, respectively. Fish fed diet D4 (Pro-Pac+Imo-Zin) showed the highest serum albumin, globulin and total protein content while control group showed the lowest serum of these indices. In the same trend levels of ALT and AST ranged between 17.50 to 25.80 and 18.50 to 28.50 u ml^{-1}, respectively and the differences among ALT and AST levels were significant (Table 3). Generally, all feed additives (Pro-Pac, Imo-Zin) relatively improved liver function as observed by reduction in the levels of each of ALT and AST.

Table 3. Effect of probiotic, prebiotic and their combination on hematological parameters *O. niloticus*.

	D1	D2	D3	D4	SE	*P Value*
RBCs (10^6/mm^3)	1.69 ^{b}	2.01 ^{a}	2.04 ^{a}	2.12 ^{a}	0.17	0.001
Hb%	9.01 ^{b}	11.72 ^{a}	11.30 ^{a}	12.70 ^{a}	0.29	0.022
Htc (g dl^{-1})	23.01 ^{b}	27.72 ^{a}	26.45 ^{a}	28.86 ^{a}	0.33	0.001
Albumin (g/dl)	1.68 ^{a}	1.38 ^{b}	1.41 ^{b}	1.55 ^{b}	0.12	0.001
Globulin (g/dl)	0.93^c	1.59 ^{a}	1.32 ^{a}	1.67 ^{a}	0.09	0.021
Total protein(g/dl)	2.61^b	2.97^{ab}	2.73 ^{b}	3.24 ^{a}	0.13	0.003
ALT u ml^{-1}	25.80 ^{a}	20.00 ^{b}	17.80 ^{c}	17.50 ^{c}	0.67	0.001
AST u ml^{-1}	28.50 ^{a}	20.15 ^{b}	18.60 ^{b}	18.50 ^{b}	0.17	0.003

Means followed by different letters in each row for each trait were significantly ($P<0.05$) different.

Proximate composition of the whole fish:

Dry matter in the whole fish body ranged between 21.39 and 23.29%. Fish group fed the control diet showed the lowest dry matter content while fish fed the diet D2 showed the highest dry matter content. Protein content of fish bodies ranged between 57.40 to 61.45%, ether extract ranged between 22.29 to 26.71% and ash content ranged between 10.42 to 10.81% and the differences in dry matter, protein, ether extract and ash among all fish groups fed the different diets were significant. The highest significant ($P<0.05$) protein and the lowest ether extract were recorded for fish group fed the diet supplemented with probiotic (D2) while control group showed the opposite trend. Ash content did not significantly ($P>0.05$) affected by the three feed additives Pro-Pac or Im-Zin in D2, D3 or D4.

DISCUSSION

Growth performance:

Results in Table 2, showed that, BW, BL, WG and SGR of *O. niloticus* significantly ($P<0.05$) improved in fish groups fed all treated diets (D2, D3 and D4). Addition of the probiotic or prebiotic singly in D2 and D3 significantly improved growth performance parameters compared to control group. The largest increases in growth and feed utilization were observed in fish group fed the diet supplemented with symbiotic (D4). Improved growth performance in fish fed diets supplemented with a probiotic have also been observed in Nile tilapia, *O.niloticus* by (Soltan and El-Laithy, 2008; Mehisan *et al.,* 2015 Soltan *et al.,* 2016; Hassanien *et al.*, 2017), rainbow trout, *Oncorhynchus mykiss* (De Wet, 2005) and rohu, *Labeo rohita* (Bairagi, *et al.,* 2004). Probiotics could stimulate digestion and food absorption by enhancing the synthesis of vitamins and amino acids, increases beneficial microbes and improve intestinal microbiological balance, microbial enzymatic activity and therefore improve digestion and feed utilization (Bairagi *et al*., 2004 and Ringø, 2010).

Compared with fish fed the untreated diet (D1) fish fed the diet supplemented with Imo-Zin (prebiotic) showed a significant ($P<0.05$) increase

in BW, BL, WG and SGR of *O. niloticus*. The obtained results are in accordance with Hassaan *et al*., (2014) who found that, *O. niloticus* fed the diet containing 1.0% yeast extract showed the highest BW, BL, WG and SGR when compared to fish fed the control diet. In contrary, Jarolowicz *et al.,* (2012) indicated that, the commercial diet supplemented with yeast extract did not have an impact on the final BW of Juvenile European pikeperch, *Sander lucioperca*. The potential mechanism of prebiotics includes a selective increase/decrease in specific intestinal bacteria that modulate local cytokine and antibody production, an increase in the intestinal short chain fatty acids production, an enhanced binding of these fatty acids to G-coupled protein receptors on leycocytes, an interaction with carbohydrates receptors on intestinal epithelial and immune cells, and partial absorption resulting in a local and systemic contact with the immune system (Seifert and Watzl, 2007).

Referring to the effect of symbiotic on growth performance, the highest final BW, BL, WG and SGR were recorded by fish that were fed D4 containing the combination of Pro-Pac and Imo-Zin (probiotic+prebiotic) compared to control and the other two fish groups (D2 and D3). Hassaan *et al.,* (2014) indicated that. *O niloticus* fed the diet contained (0.48×10^6 CUF g^{-1} *B. licheniformis* and 1.0% yeast extract) gained the highest BW, WG and SGR compared with fish fed control and the diets supplemented *B. licheniformis* or yeast extract singly. Recently, Hassaan *et al.,* (2017) showed that highest values of BW, WG and SGR of *O. niloticus* fed diets supplemented with the two combinations of 5 g malic acid/kg and 1.1×10^5 cfu/g *B. subtiis* and 10 g malic acid/kg and 1.1×10^5 cfu/g *B. subtiis*.

Survival rate:

Our results showed that, incorporation of probiotic, prebiotic or their combination in the diets showed a significant improvement in survival rate of *O. niloticus*. On the other hand, there were insignificant differences in survival rate among fish groups fed the treated diets (D2, D3 and D4). Probiotic administration is associated with the enhancement of host resistance to environmental and nutritional stressors, improving survival and growth rates. Probiotics play a major

role in preventing the occurrence of diseases by producing certain inhibitory compounds that act antagonistically against the pathogenic microbes and hence, preventing their proliferation in the host bodies (Mehisan *et al.*, 2015; Hassanien *et al.*, 2017). The anti-pathogenic activity may be due to singular or combination of production of antibiotics (Williams and Vickers, 1986), bacteriocins (Panigrahi and Azad, 2007), siderophores, lysozymes, proteases, hydrogen peroxide and the alteration of pH values (Sugita *et al.*, 2009). Recently, Hassaan *et al.* (2017) investigated effect of dietary supplementation with malic acid (0.0, 5.0, and 10.0 g kg^{-1}), each of which was supplemented with 0 and 1.1×10^5 cfu g^{-1}*B. subtilis* in 3 × 2 factorial experiment on survival rate of *O. niloticus*. The results indicated that survival rates were significantly higher in all dietary treatments in comparison with control fish group.

Feed intake and feed utilization:

Results of Table 2 indicated that, compared to control diet, supplementation of the experimental diets with each of probiotic (Pro-Pac), Prebiotic (Imo-Zin) and their combination (symbiotic) significantly increased feed intake (FI) and improved FCR, PER and PPV which was subsequently followed by an increase in the growth performance and this means that the use of probiotics can decrease the amount of feed necessary for animal growth and therefore reduce production costs. Several studies on probiotics have been published in recent years and suggested that, probiotics provide nutritional benefits in diets for tilapia fingerling (Soltan and El-laithy, 2008, Soltan *et al.*, 2016; Hassanien *et al.*, 2017). Also, incorporation of prebiotic in *O. niloticus* diets significantly increased feed intake and improved protein productive value. Hassaan *et al.* (2014) noticed that increasing yeast extract levels (from 0, 0.5 or 1.0%) in *O. niloticus* diets significantly ($P<0.01$) improved FI and FCR, PER and PPV.

Referring to the dietary symbiotic effect results of the present study showed that, dietary symbiotic (Pro-Pac+Imo-Zin) showed the highest FI, the best FCR, PER and PPV compared to fish groups fed the control diet (D1) and probiotic (D2), prebiotic (D3) supplemented diets. These results were parallel to

that obtained for growth parameters (BW, BL, WG and SGR) obtained in the present study. Gastrointestinal bacteria take part in the decomposition of nutrients, provide the microorganisms with physiologically active materials, such as enzymes, amino acids, and vitamins (Wache¢ *et al*., 2006; Wang, 2007; Wang and Xu, 2006) and thus facilitate feed utilization and digestion. This may account for the enhanced FCR, PER and PPV by dietary *Lactobacillus acidophilus, L. plantarum*; *Enterococcus faecium*; *Bifidobacterium bifidum* that presented in Pro-Pac in the present study and previous studies (Bairagi *et al.*, 2004; 2008). Mehrabi *et al.,* (2012) came to similar results. They found that, dietary symbiotic produced a better significant ($P<0.05$) FCR for rainbow trout, *Oncorhynchus mykiss* fingerlings. Ye *et al.,* (2011) reported that, Japanese flounder fed diet supplemented with (FOS, MOS and *Bacillus clausii*) improved FCR than other diets. Also, Ai *et al*. (2011) showed that juvenile large yellow croaker, *Larimichthys crocea* fed the diet supplemented with FOS and *Bacillus. Subtilis* 0.96×10^{6} CFU g^{-1} significantly improved FCR and PER values when compared to fish group fed the control diet.

Hematological and liver function indices:

Supplementation of the basal diet with Pro-pac, prebiotic or symbiotic significantly increased Red blood cells (RBCs), Haemoglobin (Hb), and hematocrit (Htc) of *O. niloticus*. Hematology is an important factor that could be considered for fish diet quality assessment. Ologhobo, (1992) reported that one of the most common blood variables consistently influenced by diet are the hematocrit (Htc) and hemoglobin (Hb) levels. Probiotics and prebiotics have been used singly or together in various animals including Nile Tilapia, *O. nioticus* (Marzouk *et al.,* 2008 and Hassaan *et al.,* 2014 & 2017), rainbow trout, *Oncorhynchus mykiss* (Firouzbakhsh *et al.,* (2012) and reported positive effects on haematological parameters. On the other hand, *O. niloticus* fed diet supplemented with *B. subtilis* (Soltan and El-Laithy 2008) or supplemented with *Pediococcus acidilactici* (Ferguson *et al.,* (2010) showed some variation (but not significant) in Hb and Htc content among the control and fish that were fish groups fed diet enriched with probiotics.

Table 3 also showed that, inclusion of probiotic, prebiotic or symbiotic significantly increased serum globulin and total protein content while albumin significantly decreased. Mehrabi *et al.* (2011) reported that diet supplemented with symbiotic (Biomin IMBO) increased the serum protein, albumin and globulin level of rainbow trout.

Alanine aminotransferase (ALT) and aspertat aminotranferase (AST) enzymes are important liver enzymes. They indicators for liver health and function through controlling the transferring amino group function of alpha-amino acids to alpha-keto acids. Large amount of ALT and AST are released into animal blood, mostly during liver cell damage (Kumar *et al.*, 2011). Control fish group showed the highest ($P<0.05$) ALT and AST levels, while fish fed supplemented diets with Pro-Pac (D2), Imo-Zin (D3) or both (D4) showed a significant decrease in ALT and AST levels. Soltan and El-Laithy (2008) found that, ALT and AST levels significantly decreased when Nile tilapia fed diets supplemented with probiotics. Similarly, Wache¢ *et al.*, (2006) observed a decrease in the activity of AST, ALT and lactate dehydrogenase in *O. niloticus* after being fed with diet containing *Pseudomonas spp.* and a mixture of *Micrococcus luteus* and *Pseudomonas spp*. Similar results were also observed in *Cyprinus carpio* fed the extract of Cyanobacteria (Palikova *et al.,* 2004). Jarolowicz *et al.,* (2012) reported that juvenile pikeperch, *Sander lucioperca* that received yeast extract in their diets exhibited a significantly lower AST and ALT activity in comparison to the control group ($P<0.05$). *O. niloticus* fed the diets supplemented with symbiotic (0.96×10^6 *Bacillus licheniformis* and 0.5% yeast extract) recorded the lowest ($P<0.05$) ALT level, while the control group showed the highest ($P<0.05$) ALT level (Hassaan *et al.*,(2014). In this respect, Marzouk *et al.*, (2008) found that, *O. niloticus* fed the diets supplemented with dead *Saccharomyces cerevisae* yeast and both of live *Bacillus subtilis + S. cerevisae* revealed a significant ($P<0.05$) decrease in ALT and AST when compared to the control group that fed on probiotic-free diet.

Proximate analysis of the whole fish:

Chemical analysis at the end of a feeding trial is frequently used to determine the influence of feed on fish composition. According to Hepher

(1990), endogenous factors (size, sex and stage of life cycle) and exogenous factors (diet composition, feeding frequency, temperature etc.) affect the body composition of fish. It should be noted that within endogenous factors, the composition of the feed is only the factor, which could have influenced the chemical composition of fish, as other endogenous factors were maintained uniform during the study.

In the present study, the highest significant ($P<0.05$) protein and the lowest ether extract were recorded for fish group fed the diet supplemented with probiotic (D2) while control group showed the opposite trend. Ash content did not significantly ($P>0.05$) affected by the two feed additives Pro-Pac or Im-Zin in D2, D3 or D4 (Table 4). The highest body protein content in *O. niloticsu* whole bodies implies on this fact that by the application of symbiotics, the ingested food was converted more effectively into the structural protein and subsequently resulted in more muscle, which is a desirable aspect in fish farming. Soltan and El-laithy (2008) indicated that, *O. niloticus* fed the diet supplemented with *B. subtilis* recorded a high level of dry matter and lipid content than control group. Bagheri *et al.* (2008) reported that, application of 3.8×10^9 CFU g^{-1} of *Bacillus spp*. in diet of rainbow trout fry made a significant increase in fish body protein content. Hassaan *et al.* (2014) showed that, *B. licheniformis* supplemented diets significantly ($P<0.05$) increased dry matter, lipid and protein content while ash content was not significantly affected. On the other hand, increasing yeast extract from 0 to 1.0% did not significantly alter crude protein, dry matter, lipid or ash content. Ye *et al.* (2011) in Japanese flounder showed an increase in the body protein content in fish fed a FOS, MOS and/or *B. clausii*-containing diet when compared to the control, body lipid content demonstrated an opposite trend to body protein content.

Table 4. Effect of probiotic, prebiotic and their combination on chemical composition of the whole body *O. niloticus*.

	D1	D2	D3	D4	SE	P value
moisture	78.61 [a]	76.71 [c]	77.78 [b]	77.38 [b]	0.54	0.012
Crud protein	57.40 [b]	61.45 [a]	53.60 [c]	56.00 [b]	0.44	0.023
Lipids	26.71 [a]	22.29 [b]	24.84 [a]	24.04 [a]	0.35	0.013
Ash	10.42	10.55	10.81	10.70	0.24	0.060

Means followed by different letters in each row for each trait were significantly ($P<0.05$) different.

CONCLUSSION

The results of the present study clearly indicated that the supplementation of *O. niloticus* diets with the commercial probiotic (Pro-Pac), prebiotic (Imo-Zin) singly or in combination not only enhanced the growth performance and feed utilization of Nile tilapia, but also hematological and biochemical blood parameters.

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تأثير البروبيوتيك، البريبايوتكس ومزيجهما على أداء النمو، وظائف الكبد وقياسات الدم على البلطي النيلي

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الملخص العربى

أجريت التجربة لمدة ١٢ أسبوع لدراسة تأثيرالبروبيوتيك، بريبايوتيك ومزيجهما على النمو، واستخدام الأعلاف، وظائف الكبد وكذلك بعض قياسات الدم والتحليل الكيماوى لسمكة البلطى النيلى. تم تركيب العلائق والتى قسمت إلى ٤ أقسام العليقة الأولى:عليقة المقارنة الأساسية والتى تركت بدون أى إضافات،العليقة الثانية: العليقة الأساسية مضافاً إليها البروبيوتيك بمعدل ١٥جرام/كجم،العليقة الثالثة: العليقة الأساسية مضافاً إليها البريبيوتيك بمعدل ١٥جرام/كجم والعليقة الرابعة: العليقة الأساسية مضافاً إليها خليط من البروبيوتيك والبريبيوتيك (بروباك+ايموزين) بمعدل ٧.٥جرام/كجم لكل منهما. تم توزيع متوسط الاصبعيات ٣.١٧ ± ٠.٠٥ جرام عشوائيا على ١٢ خزانا من فيبر جلاس (٢٠٠ لتر) وكل حوض يحمل ٢٠ سمكة وتم توزيعها عشوائيا على ثلاث مكررات غذيت الأسماك على العلف مرتين يوميا في الساعة ٩:٠٠ صباحا و٣:٠٠ مساء.

بعد ١٢ أسبوعا، أظهرت الأسماك التي تغذت على البروبيوتيك (D2)، بروبيوتيك (D3) أو مزيجهما (D4) أعلى وزن الجسم (BW)، وطول الجسم (BL)، وزيادة الوزن (WG) معدل النمو النسبى (SGR)، كمية العلف المأكول (FI)، معدل التحويل الغذائى (FCR)، نسبة كفاءة البروتين (PER)، القيمة الإنتاجية للبروتين (PPV) مقارنة بمجموعة الأسماك التى تم تغذيتها على العليقة الأساسية (المقارنه) بدون إضافات. في نفس الاتجاه، سجلت أعلى خلايا الدم الحمراء (**RBCs**)، الهيموجلوبين (**Hb**)، الهيماتوكريت (**Htc**)، الجلوبيولين والبروتين الكلي للأسماك التى تم تغذيتها على العلائق المحتوية على الإضافات المختلفة مقارنة بمجموعة الأسماك التى تم تغذيتها على العليقة الأساسية (D1). من ناحية أخرى، حققت الأسماك التى غذيت على عليقة المقارنة (العليقة الأولى) أعلى مستوى لإنزيمات الكبد (ALT & AST) كما ادت إضافة منشطات النمو الحيوية (البروبيوتيك، البريبيوتيك، وخلطات هذه الإضافات) إلى خفض مستوى هذه الإنزيمات فى الدم. تم تسجيل أعلى نسبة للأسماك التى تم تغذيتها على عليقة المقارنة أعلى نسب للمادة الجافة واللبيدات واقل محتوى من الرماد. وفى المقابل حققت مجموعة الأسماك التى غذيت على العليقة الثانية والمحتوبة على البروبيوتيك أعلى نسبة للبروتين الخام.