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Effect of adding *Pediococcus acidilactici* at low plant protein diets on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings

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ABSTRACT

In the present study effects of adding the probiotic *Pediococcus acidilactici* (PA), on growth performance, feed utilization and carcass composition of Nile tilapia, (*Oreochromis niloticus*) fingerlings were investigated. Four experimental diets were formulated, as isocaloric (4417.23 Kcal/kg diet), containing 25% crude protein (CP), and 23% crude protein (CP), each was either addition or not with *Pediococcus acidilactici* (PA) at 1g /kg diet (T1, T2, T3 and T4), respectively and fed to monosex Nile tilapia, (*Oreochromis niloticus*) fingerlings with average weight (2.60 ±0.01g/fish). Total of 240 fingerlings were random distributed in four treatments, triplicate groups each with twenty fish/ aquarium. The experiment period was lasted for 71 days.

The results indicated that fish fed low level of crude protein (23%) in tilapia diet without *Pediococcus acidilactici* recorded the worst values of growth performance and feed utilization parameters. Also, fish fed with *P. acidilactici* presented higher weight gain and specific growth rate (SGR) and the best feed conversion ratio (FCR), protein efficiency ratios (PER), protein productive value (PPV %) and energy utilization compared to the control diet. The results indicated that the use of 1×10^9 cuf kg-1 dry feed probiotics improved growth parameters and feed utilization in Nile tilapia fingerlings.

Keywords: probiotics, *Pediococcus acidilactici*, growth performance, feed utilization, Economic evaluation, plant protein diet, *Oreochromis niloticus.*

INTRODUCTION

The optimum protein level for Nile tilapia (*O. niloticus*) has been the aim of many research studies in order to increase farm profitability. A wide range of 25-56% dietary crude protein level has been reported to be the protein level inducing maximum weight gain (El-Dahhar, 1994 and Wu *et al*., 1995). Variation in protein requirements is due to different reasons; fish size, feeding rates, environmental conditions, protein and energy quality and their concentration in the diet (Lovell, 1989). Ogunji and Wirth (2002) concluded that a dietary deficiency in protein results not only in a deficiency of essential amino acids in the body but also affects transport and storage of lipids within the fish body. It is well known that feed additives can be used safely in fish ration to improve their performance. Meanwhile, feed additives when added to diets in very small quantities led to better performance.

Probiotics produce a variety of organic acids and products, such as volatile fatty acids and lactic acid as a part of their metabolism of nutrients in the gut digest (Gibson, 1999). The weak organic acids cause lower the pH of the gut environment that essential for the survival of pathogenic (Marteau *et al*., 2004). The action mechanism of probiotics might include:- reduction of toxin production; stimulation of enzyme production by the

host; production of some vitamins or antimicrobial substances; competition for adhesion to epithelial cells and increased resistance to colonization; stimulation of immune system of the host and reduction of fish stress (Falcao *et al*., 2007; Shehata and Tawfeek, 2010). Probiotics have no critical problems with the thermal processing of the food and acid conditions of the stomach and safety, which not introduce foreign microbial species into the gut (Forchielli and Walker, 2005).

Pedicoccus acidilactici is a product from lactic acid bacteria (LAB) and it is a probiotics. It is a live harmless bacterium that helps the well-beings of the host animal and contribute, directly or indirectly to the host animal against harmful bacterial pathogens, when consumed in adequate amounts, production of antagonistic compounds against pathogens (Ferguson *et al*., 2010). The supplementation of bactocell (*Pedicoccus acidilactici*) at 1gm/kg diet of Nile tilapia and Common carp has been found to improve fish immunity, livability, body weight gain and decrease the mortality level with improvement the productive and economic efficiency of fish farms (EL-Banna and Atallah, 2009).

Meanwhile, (Sara *et al*., 2016) revealed that the role of *P. acidilactici* in the diet of Oriental bream fry (*Abramis brama orientalis*), *achieved increasing* growth performance and improved feed efficiency ratio. Sabreen *et al*., (2013) found that when used large doses of *P.acidilactici* (2, 3 gm/kg diet) showed high mortality rate (58.3%) that may be regarded to decreased immunity due to using large dose prolonged and continuous use of probiotics which lead to simultaneous stimulation of spleen. This suggestion was confirmed by histological changes in spleen where spleen showed marked lymphoid depletion, (Bricknell and Dalmo 2005). On the other hand, Castex *et al*., (2008) observed that, when used *P.acidilactici* as a continuous feed additive for long period with leads to adverse effect on shrimp. Also, Venkat *et al.* (2004) showed that strains of *Lactobacillus acidophilus* and *L. sporogenes* significantly improved growth of *Macrobrachium rosenbergii* post larvae, and the same authors observed inhibitory effects of the LABs tested against the gram-negative flora present in the fresh water of the shrimp's gut.

Regarding the effect of interaction between dietary CP and probiotics, Lara-Flores *et al.* (2003) evaluated the effects of probiotics on growth performance in Nile tilapia under two stress factors, the dietary protein level and stocking density. They found that the fry fed diets with a probiotic supplement exhibited greater growth than those fed the control diet without probiotic.

The aim of this research was investigated the effect of adding *Pediococcus acidilactici* at low plant protein diets on growth performance of mono sex Nile tilapia *(Oreochromis niloticus)* fingerlings.

MATERIALS AND METHODS

The present study was carried out at the Utilization By-products Department, Animal Production Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Fish and culture facilities:

Mono sex Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from private tilapia hatchery, at Ibshaway Center, Fayoum Governorate, Egypt. The average initial body weight of Nile tilapia fingerlings was (2.59 ± 0.01g). The fish fingerlings were acclimated to the laboratory conditions for 2 weeks in 1m3 fiberglass tank and fed commercial diet containing (25 and 22%) crude protein. Before starting the experiment, a batch of fish was randomly selected, weighed and stored as zero group at -4°C for proximate chemical analysis thereafter. The fish were stocked at a density of 20 fish / aquarium (70 l each) in duplicates via water recalculating system. Water exchange rate of the system was approximately 10% of

total volume per day. All fish were fed their respective diets at a level of 6% of body weight for 13 days biweekly. The daily ration was divided into two equal portions and offered two times a day at 7.00 and 14.00 hrs. The fish in each replicate were weighed biweekly at the 14th day and the amount of daily diet was adjusted accordingly. The actual experimental period extended for 71 days.

Experimental diets.

Table (1): Proximate analysis (DM %) of the feed ingredients used in formulating the experimental diets fed to Nile tilapia, (*Oreochromis niloticus*) fingerlings

Ingredients	Moist.	Crude protein	Ether Extract	Crude Fiber	Ash	NFE*	GE**
Soybean meal	8.85	44.0	1.49	7.19	6.23	41.09	4558
Wheat bran	10.78	13.73	3.35	11.62	7.11	64.19	4222
Yellow corn	11.00	7.50	3.80	2.60	1.30	84.80	4280
Corn gluten	9.4	60.42	2.04	1.36	1.280	34.90	5051
Cora oil	-	-	-	-	-	-	8000

* Calculated by difference.,

**Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, 4.0 and 4.0 (*Kcal* GE/Kg DM) for crude protein, ether extract, crud fiber and nitrogen free extract, respectively (Jobling, 1983).

Table (2).Diets formulation and proximate analysis (%) of the experimental diets fed to Nile tilapia, (*Oreochromis niloticus*) fingerlings.

Ingredients (%)	Г1 (25% CP) (Control)	T2 (25% CP + PA)	T3 (23% CP)	T4(22%CP +PA)
Soybean meal (44%CP)	30.65	30.65	30.65	30.65
Yellow corn	46.20	46.10	50.20	50.10
Corn gluten meal	12.00	12.00	8.00	8.00
Wheat bran	6.50	6.50	6.50	6.50
Corn oil	2.00	2.00	2.00	2.00
Vit and Min. Mix[1].	1.00	1.00	1.00	1.00
Di Ca phosphate	0.5	0.5	0.5	0.5
DL- lysine	0.5	0.5	0.5	0.5
DL- methionine	0.2	0.2	0.2	0.2
Calcium carbonate	0.45	0.45	0.45	0.45
Pediococcus acidilactici (PA)	-	0.10	-	0.10
Total	100.00	100.00	100.00	100.00
Proximate analysis (%)on DM basis				
Dry mater	88.99	88.84	89.03	89.16
Crude Protein	25.09	25.02	23.36	23.28
Ether Extract	3.40	3.61	3.20	3.29
Ash	4.04	3.99	3.94	4.14
Crude Fiber	5.38	5.72	5.11	5.33
Nitrogen free extract[2]	62.06	61.66	64.39	63.93
GE[3] (kcal\kg)	4432.26	4445.53	4398.25	4392.33
Ca	0.40	0.40	0.40	0.40
P	0.52	0.52	0.51	0.51
Lysine	1.53	1.53	1.50	1.50
Meth.	1.13	1.13	1.10	1.10

[1]Vitamin and Mineral mixture/kg premix containing the following: 3300 IU vitamin A, vitamin D3, 410 IU vitamin E,2660 mg vitamin B1,133mg vitamin B2,580 mg vitamin B6 ,410 mg vitamin B12- 50 mg biotin , 9330 mg Colin chloride,4000mg vitamin C, 2660 mg Inositol, 330 mg para -amino benzoic acid, 9330 mg niacin, 26.60 mg pantothenic acid.and 325 mg Manganese, 200mg Iron,25 mg Copper, 5 mg Iodine, 5mg Cobalt. [2]Calculated by difference. [3]GE : Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, 4.0 and 4.0 (*Kcal* GE/Kg DM) for crude protein, ether extract, crud fiber and nitrogen free extract, respectively (Jobling, 1983).

All feed ingredients and the necessary additives are purchased from the local market. The proximate analysis of the feed ingredients used in formulating the experimental diets is shown in Table (1). Almost isocaloric (4417±26 Kcal GE) four experimental diets were formulated in the present experiment (Table 2). The tested commercial probiotic *Pediococcus acidilactici* (PA) being (25 and 22%) crude protein (T2 & T4).

Two basal diets were formulated to contain the recommended 25% CP (T1) and low crude protein level of 23% CP (T3). *Pediococcus acidilactici* (PA) was added to the experimental diets (T2 and T4) at 0.1 g/kg for the basal diet as Bactocell ® which 1g of commercial product contains 1x109 CFU, as recommended by Biotal Company, UK. Most of the plant protein sources are deficient in certain essential amino acids, and may therefore lead to retarded performance (Francis *et al.*, 2001). Supplementing these sources with certain compounds may improve their nutritive values for fish. However, Lysine and methionine were added to the diets to adjust the amino acids required by Nile tilapia according to (NRC 1993). The four formulated diets were separately processed by blending the dry ingredients into a homogeneous mixture, added 10% warm water and then passing the mixed diet through a laboratory pellet mill with diy 2mm. The pelleted diets were dried in oven at 65°C overnight. Diets were kept in black plastic bags then stored in a refrigerator at 1°C throughout the whole experimental period.

Analytical methods:

At the beginning, from the batch of collected fish, 30 fish were analyzed for initial carcass composition.

At the end of the experiment, fish in each aquarium were meted, weighed and frozen at -20°C for final body composition analysis. All fish samples were minced homogenized with Ultra-Tunax devic. The homogenized samples were oven dried at 60 - 80°C for 48 hrs. Proximate analyses of whole body moisture, protein, fiber, lipid, and ash performed according to the methods of A.O.A.C. (2000), while nitrogen free extract (NFE %) was calculated by difference. Gross energy (Kcal GE/Kg) contents of all the samples were calculated according to Jobling, (1983). Calcium (Ca), phosphorus (P), lysine (Ly) and methionine in ingredients used were calculated according to (NRC, 1993). Water quality parameters were analyzed according to APHA (1992).

Measurements of growth and feed utilization:

The total weight gain, average daily gain, specific growth rate, feed conversion ratio, protein and energy utilization were calculated as:

1-Total weight gain (g/fish) = (WF-WI)
Where: WF, Average of final weight (g) and
WI: Average of Initial weight (g)

2- ADG (Average daily gain, g/fish/day) = total gain / duration period

3- SGR (Specific growth rate, % / day) = 100 × (ln WF- ln WI) /n.
Where: ln, Natural log and n is the duration period.

4-Feed conversion ratio (FCR) = dry matter intake (g) / total gain (g)

5- Protein productive value (PPV %) = (PT – PI) ×100/protein intake (g)
Where: PT, Protein content in fish carcass at the end and PI, Protein content at the start.

6-Energy utilization (EU%) = (ET–EI) ×100 / Energy intake (kcal)
Where: ET, Energy in fish carcass (kcal) at the end and EI, Energy in fish carcass (kcal) at the start.

Blood samples:

Blood samples were collected at the start of the experiment and at the end of the experimental period from caudal vein in dry sterilized centrifuge tube, and allowed to clot at room temperature. Samples were then centrifuged to separate serum, which were kept at-20°C till used. Aspartate aminotransferas (AST) and

alanine aminotransferas (ALT) activities were determined according to Reitman and Frankel (1957). Uric acid and creatinine were determined according to Schultz (1984), Husdan and Repoport (1968), respectively. Total protein (TP), albumin and globulin were determined according to Henry (1964) and Drupt (1974), respectively.

Analysis of gut microflora:

Fish were randomly selected from each pond, collected in sterile plastic bags, and carried to laboratory for further biological analysis. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish. Add 12-15 ml plate count agar (cooled to 45 ± 1°C) to each plate within 15 min of original dilution. For milk samples, pour an agar control, pour a dilution water control and pipet water for a pipet control. Add agar to the latter two for each series of samples. Add agar immediately to petri dishes when sample diluent contains hygroscopic materials, e.g., flour and starch. Pour agar and dilution water control plates for each series of samples. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar solidify. Invert solidified petri dishes, and incubatepromptly for 48 ± 2 h at 35°C. Do not stack plates when pouring agar or when agar is solidifying (Larry and James, 2001; Paludan- Muller *et al*., 1999).

Statistical methods:

The collected data were subjected to one-way analysis of variance (ANOVA) using SAS procedure (SAS, 1993). Duncan's multiple rang test Duncan, (1955) was used to compare differences among individual means. Treatment effects considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Water quality :

All values of the water quality parameters in the present experiment were in the normal range for rearing Nile tilapia as indicated by the following figures obtained quality: Temperature (28 ± 0.5°C), dissolved oxygen was measured at 7.5h (6.3 ± 0.4 mgl^{-1}), total ammonia (0.089 ± 0.11 mgl^{-1}), nitrite (0.04 ± 0.01 mgl^{-1}), and pH (8.7 ± 0.12). It is noticed that through the periodical examination of determined water quality parameters were within the acceptable range for Nile tilapia growth (Stickney, 1979).

Growth performance:

The effects of *Pediococcus acidilactici* (PA) additive on growth performance of Nile tilapia fingerlings were showed in Table (3). The initial body weight of all used fish was almost similar, which confirmed appropriate randomization process. Growth performance measured as final weight (g/fish) were not significantly differed between all treatments (P>0.05) compared with fish fed diet T3.

Table (3). Effects of *Pediococcus acidilactici* (PA), additives on growth performance of Nile tilapia, (*Oreochromis niloticus*) fingerlings.

Exp. Diets	Live weight (g/fish)		Weight gain (g/fish)	SGR (%/day)
	Initial	Final		
T_1(25% CP)	2.60 ± 0.01	8.65^{a} ±0.32	6.05^{a} ±0.32	1.69^{a} ±0.05
T2(25%CP + PA)	2.59± 0.01	9.46^{a} ±0.07	6.87^{a} ±0.07	1.82^{a} ±0.01
T3(23% CP)	2.60± 0.01	7.56^{b} ±0.47	4.97^{b} ±0.47	1.50^{b} ±0.09
T4(23%CP + PA)	2.59± 0.01	8.84^{a} ±0.17	6.25^{a} ±0.17	1.73^{a} ±0.03

a,b: Mean bearing the same letters within each column do not differ significantly (P>0.05).

Data in Table (3) indicated that the best weight gain and SGR were observed with fish group fed T2 followed by T4 and T1 (P>0.05) and decreased significantly in fish group fed low crude protein (T3). It means that *P. acidilactici* (PA) added to Nile Tilapia diets enhancement the weight gain and SGR even with low protein requirement comparable to the fish fed diet with recommended crude protein without *P. acidilactici* (PA) additive. These results were agreed with ones obtained by El-Haroun *et al.,* (2006), who reported that the improvement of growth by using probiotics is related to an enhancement of nutrition, as some probiotic strains may serve as a supplementary source of food and their activity in the digestive tract consequently be a source of essential nutrients (Balcazar *et al*., 2006).

Feed and nutrient utilization:

The effects of *Pediococcus acidilactici* (PA) additives on feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV%) and energy utilization (EU%) of *O. niloticus* fingerlings were presented in Table (4). It was observed that fish group fed the recommended level of crude protein with adding *Pediococcus acidilactici* PA (T2) has decreasing figure of feed intakes (P<0.05) compared to (T1) without adding the tested probiotic. The same trend was resulted by fish group fed low protein content plus *Pediococcus acidilactici* PA (T4) compared with T3 (14.58 and 12.89), respectively.

Table (4).Effects of *Pediococcus acidilactici* (PA), additives on feed and nutrient utilization parameters of Nile tilapia, (*O. niloticus)* fingerlings

Exp. Diets	Feed utilization		Protein utilization	EU%	
	Feed intake(g/fish)	FCR	PER	PPV%	
$T_{1(25\% CP)++}$	13.70^{ab} ±0.46	2.28^{b} ±0.14	1.48^{b} ±0.09	28.10^{bc} ±1.29	17.26^{bc} ±0.77
$T2_{(25\%+ PA)}$	12.47^{c} ±0.07	1.82^{b} ±0.03	1.84^{a} ±0.02	36.96^{a} ±2.91	21.52^{a} ±1.29
$T3_{(23\% CP)}$	14.58^{a} ±0.39	2.99^{a} ±0.34	1.14^{c} ±0.12	22.85^{c} ±2.15	13.67^{c} ±1.22
$T4_{(23\%+ PA}$	12.89^{bc} ±0.10	2.07^{b} ±0.05	1.62^{ab} ±0.05	32.55^{ab} ±1.66	19.25^{ab} ±1.06

a,b: Mean bearing the same letters within each column do not differ significantly(P>0.05).

Furthermore, insignificantly increasing in feed intakes was estimated in fish group fed low protein content (T3) compared with (T1). The best-feed conversion ratio (FCR) was recorded with fish fed T2, T4 followed by T1 without adding the tested probiotic (P > 0.05). However, T3 (23% CP + PA), group had the worth value of FCR with significant differences (P < 0.05) compared to the other treatment groups. The fish fed T2 and T4 diets (addition of *Pediococcus*

acidilactici) had insignificant (P > 0.05) higher values of PER, PPV and EU than the control diets. However, PER, PPV and EU for fish fed T3 diet recorded the lower value (1.14, 22.85 and 13.67%, respectively) compared to the other treatment groups. The improvement may be a result of the ability of *Pediococcus acidiolactici* to keep the integrity of intestine making it more able to absorb nutrients (Sara *et al.,* 2016). In addition, *Pediococcus acidiolactici* are considered as an added nutritive value for being a rich source of protein and vitamin B-complex (Shehata and Tawfeek, 2010).

Carcass composition of fish:

The whole body composition of the fish fed the experimental diets are presented in Table (5). There were no significant differences in dry matter, lipid and energy content of the fish. In addition, ash content was insignificantly higher at the start of experiment than the end of experiment. Meanwhile, the fish fed T2 (25%CP + PA) diet has significantly higher crude protein content than the other treatments. The uppermost two values (54.96 and 53.19%) of crude protein were achieved for fish fed diets T2 and T1 respectively, with no significant difference (P > 0.05). Lara-Flores *et al.,* (2003) reported that diets containing probiotics could not significantly affect the body composition of Nile tilapia.

Table (5). Effects of *Pediococcus acidilactici* (PA), additives on carcass composition of Nile tilapia, (*O.niloticus)* fingerlings

Exp.diets	Dry Matter %	CP %	EE %	Ash%	Gross energy (kcal/kg)
At the start	29.88±1.88	51.29^{b} ±1.72	26.77±2.34	9.91±1.11	895.47±127.15
At the end					
$T_{1(25\% CP)}$	27.56±0.62	53.19^{ab}±0.42	27.04±0.53	9.28±0.05	5966.55±33.73
$T2_{(25\% +PA)}$	27.53 ±0.96	54.96^{a}±0.70	25.97±0.52	8.69±0.10	5961.39±12.27
$T3_{(23\% CP)}$	27.99±0.29	51.83^{b}±0.54	27.05±0.70	8.76±0.54	5965.81±18.01
$T4_{(23\% + PA)}$	27.86±0.35	51.77^{b}±0.66	26.03±0.15	8.50±0.12	5919.83±7.34

a,b: Mean bearing the same letters within each column do not differ significantly (P>0.05)

Several studies on probiotics in aquaculture have used in vitro models of specific bacteria as antagonists of pathogens (Vine *et al.,* 2006). Other important effect of the use of probiotic, that it is not extensively study, but demonstrated an important effect, of the feed efficiency and the growth promotion (Lara-Flores *et al.,* 2003, 2010). Probiotics are biopreparations containing living microbial cells that optimize the colonization and composition of the growth and gut micro flora in animals, and stimulate digestive processes and immunity (Bomba *et al.,* 2002). The results of the current study confirmed that the incorporation of *Pediococcus acidilactici* (PA) in the diets containing low protein was superior to the corresponding diet with the same CP level but without supplementation of (PA). This may be due to the effect of the tested probiotics, which improved absorption of nutrients, and depressed harmful bacterial affects that cause's growth depression. Similar results were observed by Fernandes and Shahani (1990), who indicated that probiotic preparations contain multiple strains of *Lactobacillus*, which are highly active against a wide range of stress conditions in the fish gastrointestinal tract, resulting in higher immunity and higher rate of utilizing nutrients and accordingly higher growth rate. In this connection, Nikoskelainen *et al.,* (2001) obtained better growth response

with diets supplemented with probiotics containing bacteria. *Pediococcus acidilactici* is a probiotic bacterium that presents positive effects on the balance and the role of the intestinal flora; it also enhance fish immunity, and improves the production performances of animals (Stella *et al.,* 2005). The probiotic, after transit thought the stomach, they attach in the intestine and use a large number of carbohydrates for their growth and produce a range of relevant digestive enzymes (amylase, protease and lipase), that increase the digestibility of organic matter and protein, produce a higher growth, prevent intestinal disorders and produce or/and stimulate a pre-digestion of secondary compounds present principal in plant sources (Lara-Flores *et al.,* 2010). Moreover, the nutritional benefits of probiotic bacteria have been attributed to the synthesis of B vitamins and short chain fatty acids in the intestine, and the higher availability of trace elements (Holzapfel *et al.,* 1998).

Table (6). Effects of *Pediococcus acidilactici* (PA), additives on blood Parameters of Nile tilapia, (*Oreochromis niloticus*) fingerlings

Item	Treatments				
	T_1 (25% CP) control	T2 (25% CP+ PA)	T3 (23% CP)	T4 (23% CP+ PA)	Start time
ALT (Iu/L)	19.67^{a} ±1.67	15.33^{bc} ±0.88	13.67^{c} ±0.67	17.67^{ab} ±1.20	17.00^{abc}±0.43
AST (Iu/L)	22.33±1.45	21.00 ±1.00	19.33 ±0.33	19.67 ±0.33	20.00±1.01
UA (mg/dl)	2.47^{a} ± 0.27	2.03^{ab}± 0.03	1.97^{ab}± 0.03	1.70^{b} ± 0.10	1.90^{ab}± 0.35
Creat. (mg/dl)	1.00 ± 0.06	0.87 ± 0.03	0.87 ± 0.03	0.90 ± 0.06	0.90 ± 0.12
TP. (g/dl)	4.43 ± 0.7	4.70 ± 0.10	5.10 ± 0.06	5.07 ± 0.12	4.60 ± 0.64
Alb. (g/dl)	2.97 ± 0.03	3.10 ± 0.06	3.30 ± 0.06	3.37 ± 0.09	2.90 ± 0.64
Glob. (g/dl)	1.46 ± 0.06	1.60 ± 0.04	1.80 ± 0.01	1.70 ± 0.03	1.70 ± 0.03

a, b, c; means within the same row with different superscript are not significantly (P>0.05).
Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Uric acid (UA), Creatinine (Creat), Total protein (TP), Albumin (Alb) and globulin (Glob).

Biochemical parameters:

Results in Table (6) showed that, the mean values of the liver and kidney enzymes (ALT and AST for liver and urea and creatnin for kidney), the serum ALT and AST activities increased significantly in fish at the start of the experiment and the control diet (T1). Meanwhile, the addition of *P. acidilactici* (PA) insignificantly decreased AST and significantly in ALT values compared with the control, while the lowest values of ALT and AST were in diet T3 (23% CP) .

Uric acid levels (UA) in fish fed diet T4 containing (22%CP + PA) decreased significantly compared with the control diet (T1), meanwhile the highest insignificant uric acid (2.47 mg/dl) was obtained by fish fed diet T1 followed by diet T2 (2.03 mg/dl). There were not significant variations of Creatinine (Creat.) in fish at the start of the experiment and the other group. These results may be due to the immuno-modulatory effect of *P. acidilactici* on the liver cells activating the anabolic capacity to produce blood proteins particularly globulin. These results closely met those of Marzouk *et al.*, (2008). Another explanation for the obtained results is the antioxidant effect of *P. acidilactici* where all living organisms are under constant attack from free radicals, which can lead to serious cellular damage if produced in excess (Castex *et al.,* 2010). On the other hand, results of protein profile which showed insignificant increase (P > 0.05) in total protein and albumin in all groups. This increased non-specific immune response may be due to the ability of the probiotics to increase the nutritional value of ration, modify the fish associated gut microbial community. More added, probiotic applications were shown to improve intestinal microbial balance,

thus leading to improve food absorption (Bairagi *et al.,* 2002; Gutowska *et al.*, 2004). Probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by the breakdown of indigestible components (Irianto and Austin, 2002).

Table (7). Effects of *Pediococcus acidilactici* (PA), additives on Total aerobic Bacterial Counts (TBC) of Nile tilapia, (*Oreochromis niloticus*) fingerlings.

Item	Treatments			
	T1 (25%CP) control	T2 (25%CP+ PA)	T3 (23%CP)	T4 (23% CP+ PA)
Total Bacterial Counts (TPBC)	$9.67x10^1$ cfu/gm	$12.33\ x10^1$ cfu/gm	$37.17x10^1$ cfu/gm	$44.21x10^1$ cfu/gm

The total bacterial load of gut sample in Nile tilapia was analysed at the end of the experimental, as explained in Table (6). The populations of the total bacterial in the intestinal tracts of the fish group fed diets with adding PA (T2) (9.67x101cfu/gm) and (T4) (37.17x101cfu/gm) respectively, were higher than those for the diets (T1) (12.33x101 cfu/gm) and (T3) (44.21x101cfu /gm) respectively, without adding the tested probiotic. The bacterial population in the gut of the fish generally varies due to the hydrobiological fluctuations occurring in the natural systems (Rheinheimer, 1985). Probiotics are live microbial cells that are administrated to intestinal tract as a feed supplement and improving its intestinal microbial balance and health (Fuller, 1989). Similarly Jatobá *et al.* (2008) supplemented tilapia with *L. plantarum* and they observed higher number of lactic acid bacteria in supplemented tilapia. These values were lower than those found by Standen *et al.* (2013) and this could be explained by the low concentration of bacteria in the intestinal tract due to pellet leaching in the water or adverse conditions of the intestinal tract like reduced pH in the stomach, digestive enzymes and bile salts or by the method used in the probiotic addition to the diet. The general effects of probiotics on increased production and increased resistance to stress in fish and other aquatic animals in aquaculture have been confirmed to aid in the digestion of food and the absorption of vitamins, help stimulate the immune system, and break down cellulose and other polysaccharides (Sun *et al*., 2011; Mahmoudzadeh *et al.,* 2016). The present study indicated clearly that the supplementation of *P. acididlactici* enhanced not only the growth performance, but also the non-specific immune responses. This might be due to that the groups of *P. acididlactici* in the gastrointestinal tract might have promoted the growth of other gut bacteria, which reduce the Pathogen Bacterial.

Table (8). Cost of feeds required for producing one Kg gain of (*O. niloticus*) Fingerlings fed the experimental diet.

Item	T1	T2	T3	T4
Cost/kg feed(L.E)	5.88	5.93	5.56	5.60
Feed intake per Kg gain (FCR)	2.28	1.82	2.99	2.07
Feed cost /1Kg fish gain (L.E)	13.41	10.79	16.61	11.59
Percentage change in feed cost to produce one kg fish gain	100	89.46	123.86	86.43

Local market price (L.E/kg) for feed ingredients used for formulating the experimental diets at the year (2017); soybean meal= 6.2 L.E; yellow corn= 3.8 L.E; wheat bran= 3.5L.E; corn gluten= 12 LE; corn oil = 14 LE; *P. acidilactici(200g)=100L.E;* Calcium carbonate= 2.5 L.E; lysine =60 LE; methionine 70 LE; vitamins and minerals mix = 15 L.E, Di calcium phosphate = 10 LE.

Economic evaluation:

Calculations of economical efficiency of the tested diets based on the cost of feed and cost of one kg gain in weight of Nile tilapia, its ratio with the control group, are shown in Table (7). The high feed costs were calculated in T2 and T1 according to the price of probiotic included in the mentioned diets. Moreover, the feed cost of the diets T3 and T4 were reduced as a logical result of decreasing the recommended protein levels. The relation between feed costs to produce one-kilogram body weight gain was recorded also; the efficiency to get one Kg body weight gain was recorded in T2 group followed by T4 and T1 groups. From economic point of view, it could be reported that using 0.10% *Pediococcus acidilactici* in Nile tilapia fingerlings diets had the highest economic efficiency being 89.46 % and 86.43%. Meanwhile, the worst value was recorded with diet containing the lowest crude protein (23%) level without probiotics being 123.86 % according to the control diet (T1). Generally, the feed cost/Kg weight gain decreased with supplementing 1g/kg diet of probiotics (*Pediococcus acidilactici*) in Nile tilapia diets.

CONCLUSION:

The present study showed that the addition of *Pediococcus acidilactici* (PA) in Nile tilapia fingerlings diets improved animal growth and mitigated the effect of stress factors, such as the low protein level in diets. *P. acidilactici* produced the best results, and it could be a good source for optimizing growth and feed utilization with improvement of productive and economic efficiency of fish farms.

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تأثير اضافة بكتريا البيدوكوكس اسيدى لاكتيسى الى علائق نباتية منخفضة البروتين على أداء النمو فى اسماك البلطى النيلي

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المستخلص

أجريت هذه التجربة لدراسة تأثير اضافة بكتريا البيدوكوكس اسيدى لاكتيسى على أداء النمو وكفاءة الأستفادة من الغذاء وتركيب الجسم ، والكفاءة الاقتصادية لآصباعيات أسماك البلطى النيلى. زعت 240 اصبعية عشوائيا من اصبعيات أسماك البلطي النيلي بمعدل عشرين سمكة لكل حوض (سعة 70 لتر) بمتوسط وزن مبدئى 02.60 ± 0.01 جرام / سمكة لفترة زمنية 71 يوم. وتم تكوين أربعة علائق تجريبية (T1 ،T2، T3، T4)على التوالى جميعها متزنة فى الطاقة الكلية 26 ± 4417 كيلو كالورى/كجم مادة جافة ، العليقة T1 (المقارنة) تحتوى على(25 % بروتين خام) والعليقة T3 تحتوى على مستوى منخفض من البروتين الخام (23% CP) وتم اضافة البروبيوتيك (البيدوكوكس اسيدى لاكتيسى) للعلائق (T2، T4) بمعدل 1جم/كجم عليقة. بعد فترة التغذية أظهرت النتائج أن العليقة المحتوية على مستوى منخفض من البروتين الخام (23% CP) بدون اضافة البيدوكوكس سجلت أقل قيم لأداء النمو وكفاءة الاستفادة من الغذاء فى حين أن الاسماك التى تم تغذيتها على العلائق المحتوية على البيدوكوكس سجلت زيادة فى الوزن ومعدل النمو النوعى وكفاءة الاستفادة للبروتين PER,PPV وأفضل معدل تحويل غذائي (FCR) مقارنا بأسماك البلطى المغذاه على العليقة المقارنة T1. أوضحت النتائج أن اضافة (1-kg CFU 10^{9} ×1) من البروبيوتيك (البيدوكوكس) فى العلائق النباتية منخفضة البروتين أدى الى تحسن أداء النمو وكفاءة الاستفادة من الغذاء لاصبعيات اسماك البلطي النيلي .