**Egyptian Journal for Aquaculture**
P-ISSN: 2090-7877
E-ISSN: 2636-3984
www.eja.journals.ekb.eg/
Nehad *et al.*, 2019; 9 (4):35-53
DOI: 10.21608/EJA.2019.47194

# Effect of Phytase and Citric Acid on Growth Performance, Feed Utilization and its Antibacterial Activity against Fish Pathogens of Nile tilapia Fingerlings

**Nehad M.[1], Eid, A. E. [1], B. A. Ali[1], Ali Wahdan[2], Enany, M.E.[2] and Asmaa S. Abd El-Naby[3]**

[1]Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.
[2]Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.
[3]Department of Fish Nutrition and Feed Technology, Central Laboratory for Aquaculture Research, Abbaasa, Abu-Hammad, Sharkia, Egypt.

Received: June 4, 2019; Accepted: June. 30, 2019 published: 2019 Vol.9 (4):35-53

## Abstract

This experiment was aimed to investigate the effect of phytase, citric acid and their interaction on growth performance, feed utilization, body composition, economic evaluation and its antibacterial activity against fish pathogens of Nile tilapia fingerlings. Nine isonitrogenous 30 % CP and isocaloric 450.27 Kcal/100g experimental diets were prepared. Phytase was added at the level 0, 1000 and 2000 FTU/kg diet while citric acid was added at the level of 0, 30 and 60 g in the experimental diets. Six hundred and seventy five of Nile tilapia fingerlings were placed randomly in twenty seven V-shaped fiber tanks 120 L capacity of water /tank, in three replicates per treatment was used in this study. Each tank stocked with twenty five fingerlings of with an average initial body weight of 12.47±0.3g /fish. Fish were acclimated to laboratory conditions for 2 weeks prior before the experimental started. Photoperiod was 12h light/ 12h dark regulate. Fish were hand-fed to satiation, two times daily for six days a week through the whole experimental period (60 days). The results indicated that the best growth performance and feed utilization of Nile tilapia fingerlings fed on D5 (1000 FTU phytase / kg + 30g citric acid/ kg) and the lowest group fed on control diet (D1). While, antibacterial activity showed that citric acid alone and citric acid with phytase had highest activity and gave wide inhibition zones against all tested and isolated bacteria which infect Nile tilapia compare with different used antibiotics. Phytase and citric acid positively affected the growth performance, feed utilization,

whole body composition and antibacterial activity against in Nile tilapia (*O.niloticus*) fingerlings. Moreover, both the supplements interacted significantly with each other for these parameters and that the fish fed on diet No. 5, containing 1000 FTU phytase / Kg with 30 g citric acid/ kg, gave the best results.

**Keyword:** phytase, citric acid, Nile tilapia fingerlings, growth performance, feed utilization, economic evaluation, antibacterial activity.

## INTRODUCTION

Soybean meal is one of source of plant protein can addition in fish diet as alternative of animal protein source **(Ng and Romano, 2013)**. Soybean meal has high content of protein and amino acid. But it contained anti-nutritional factors as phytate which reduce absorption of protein, minerals and other nutrients. So improvement of these nutrients availability depended on addition of phytase and citric acid. Phytase is enzyme which hydrolyze phytate complex **(Cao *et al.*, 2007)**. Studies have been reported improved growth, feed performance and nutrient utilization have been observed in *Cirrhinus mrigala* **(Hussain *et al.*, 2017)**, Nile tilapia **(Abo Norag *et al.*, 2018)**. Studies have been reported improve mineral digestibility in Nile tilapia **(Nwanna and Olusola, 2014).** Citric acid is another approach to break phytate complex by reducing pH. Citric acid enhances growth and nutrients utilization **(Dai *et al.*, 2018)**. Citric acid improved bone mineralization in *L. rohita* juveniles fed on soybean meal based diet **(Shafique *et al.*, 2018)**. Also, combination between citric acid and phytase has been improved growth and nutrient utilization in *L. rohita* **(Bano and Afzal, 2017)**.

Diseases outbreak is a most problem which faces aquaculture development. Feed additives play role in enhance growth performance, immunity and reduce harmful effects due to using antibiotic. Antibacterial activity test was conducted to evaluate effects of feed additives in vitro on bacteria pathogen which formed main source in diseases outbreak. This experiment was aimed to investigate the effect of phytase, citric acid and their interaction on growth performance, feed utilization, body composition, economic evaluation and its antibacterial activity against fish pathogens of Nile tilapia fingerlings.

## MATERIAL AND METHODS

The experimental work of the present study was carried out at the Department of Animal and Fish Production, Faculty of Agriculture, Suez Canal University, Ismailia- Egypt to determine the influence of the effects

of phytase, citric acid and their interaction on growth performance, feed utilization, whole body composition, economic evaluation and its antibacterial activity against fish pathogens of Nile tilapia fingerlings.

**Experimental Facility**

Nile tilapia (*O. niloticus*) fingerlings obtained from Central Laboratory for Aquaculture Research, Abbaasa, Abu-Hammad, Sharkia Governorate, Egypt were used in the present study. Six hundred and seventy five of Nile tilapia fingerlings were placed randomly in twenty seven V-shaped fiber tanks 120 L capacity of water /tank, in three replicates per treatment was used in this study. Each tank stocked with twenty five fingerlings of with an average initial body weight of 12.47±0.3g /fish. Fish were acclimated to laboratory conditions for 2 weeks prior the experimental started. Photoperiod was 12h light/ 12h dark regulate. Each fiber tanks was aerated by using small air-bumps. Aeration was continuously provided using an air blower. Settled fish wastes along with a half of fiber tanks water was siphoned daily, and replaced by well-aerated and dechlorinated tap water from a storage tank. Fish in each fiber tanks were weighted every 10 days throughout of experimental period (60 days). Dead fish were daily recorded and removed. At the end of the study, fish were individually weighed. Water temperature was thermostatically controlled throughout the experimental period.

**Experimental design**

The present experiment was conducted to investigate the effects of phytase, citric acid and their interaction on performance of Nile tilapia fingerlings. For this trial, 675 fingerlings were used in a 3×3 factorial arrangement (0, 1000 and 2000 FTU/kg phytase and 0, 30 and 60 g / kg citric acid) as table (1).

**Table (1): Experimental design**

| Different levels of phytase (FTU phytase /kg diet) | Different levels of citric acid (g citric acid /kg diet) | | |
|---|---|---|---|
| | 0 | 30 | 60 |
| 0 | D1 | D2 | D3 |
| 1000 | D4 | D5 | D6 |
| 2000 | D7 | D8 | D9 |

**Water quality parameters**

Water quality parameters (temperature, dissolved oxygen, pH, and ammonia) were monitored to ensure water quality remained well within limits recommended for Nile tilapia. Water temperature and dissolved

oxygen were measured using mettler Toledo, model 128.s/No1242 instrument Ammonia was measured by Hanna ammonia meter, every other day. pH was measured by Orion model 720A,s/no 13062 and monitored twice weekly. During the feeding trial, the water quality parameter averaged (±SD): water temperature 23.00 ± 1.00 $^{\circ}$C, dissolved oxygen 5.70 ± 0.5 mg $l^{-1}$; pH 7.7 ± 0.7 and ammonia 0.002 ± 0.007 $mgl^{-1}$.

**Experimental diet**

Table (2) showed feed ingredients (g /kg) and proximate chemical analysis (%) of the experimental diets. Nine isonitrogenous 30 % CP and isocaloric 450.27 Kcal/100g experimental diets were prepared. Phytase was added at the level 0, 1000 and 2000 FTU/kg diet while Citric acid was added at the level of 0, 30 and 60 g in the experimental diets as the following: D1 , Without any addition (control diet ); D2 , (Without any addition phytase + 30g citric acid/kg diet); D3, (Without any phytase + 60g citric acid/ Kg diet ); D4, (1000 FTU phytase /Kg diet + Without any addition citric acid); D5,(1000 FTU phytase/kg diet + 30g citric acid/kg diet); D6, (1000 FTU phytase/kg diet + 60g citric acid/kg diet), D7 (2000 FTU phytase/kg diet+ without any citric acid); D8, (2000 FTU phytase/kg diet + 30g citric acid/kg diet) and D9, (2000 FTU phytase /kg diet + 60g citric acid/kg diet . Fish in each tank were hand-fed with one of the experimental diet to satiation, two times daily (9:00 am and 2:30 pm) for six days a week through the whole experimental period (60 days).

Citric acid was obtained from (El Nasr Pharmaceutical Chemicals Company) in a powder form, minerals mixture and vitamin premix were added to ground ingredients and mixed electrically. While, phytase used was a 6-phytase (EC 3.1.3.26) obtained from *Buttiauxella sp*. expressed in *Trichoderma reesei* (Axtra PHY®, Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK). The dry ingredients of each diet were thoroughly mixed, and 100 ml of water was added per kg diet. Afterwards, the mixture (ingredients and water) was blended using a kitchen blender to make a paste of each diet. Diet ingredients were ground and thoroughly mixed and the oil was slowly added at the same time of mixing with warm water (45°C) until the diets began to clump. Dose of phytase enzyme was mixed first into warm water (Rachmawatia *et al.*, 2017) then added to feeds. Noodle-like feed pellets, which were then broken to make 2-mm die pellets, were prepared using kitchen mincer. The pellets were dried by the sun for 2 days with keeping Ventilation and flipping then stored in plastic bags when completed drying in a deep freezer at −2$^{\circ}$C until use.

Table 2: **Feed ingredients (g /k g) and proximate chemical analysis (%) of the experimental diets.**

| Ingredient | Diets No. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| **Fishmeal (60% CP)** | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| **Soybean meal (47%CP)** | 480.0 | 480.0 | 480.0 | 480.0 | 480.0 | 480.0 | 480.0 | 480.0 | 480. |
| **Wheat bran** | 120.0 | 120.0 | 120.0 | 120.0 | 120.0 | 120.0 | 120.0 | 120.0 | 120. |
| **Rice bran** | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 |
| **Yellow corn** | 220.0 | 190.0 | 160.0 | 219.9 | 189.9 | 159.9 | 219.8 | 189.8 | 159. |
| **Sunflower oil** | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| **Vitamin[1] and Minerals[2] Mix** | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| **Phytase (g)[3]** | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 |
| **Citric Acid (g)** | 0.0 | 30.0 | 60.0 | 0.0 | 30.0 | 60.0 | 0.0 | 30.0 | 60.0 |
| **Total** | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| **Proximate chemical analysis (%)** | | | | | | | | | |
| **Dry matter** | 90.44 | 90.67 | 90.59 | 90.47 | 90.74 | 90.81 | 90.77 | 90.63 | 90.4 |
| **Crude protein (CP)** | 30.05 | 30.75 | 30.25 | 30.45 | 30.30 | 30.08 | 30.30 | 30.25 | 30.4 |
| **Ether extract (EE %)** | 8.34 | 8.23 | 8.48 | 8.18 | 8.31 | 8.55 | 8.42 | 8.11 | 8.03 |
| **Total ash** | 7.42 | 7.39 | 7.47 | 7.39 | 7.34 | 7.37 | 7.03 | 7.47 | 7.27 |
| **Crude Fiber (CF)** | 5.24 | 5.62 | 5.5 | 5.91 | 5.92 | 5.6 | 5.5 | 5.74 | 5.48 |
| **NFE[4]** | 48.95 | 48.01 | 48.30 | 48.07 | 48.13 | 48.40 | 48.75 | 48.43 | 48.7 |
| **P** | 0.83 | 0.82 | 0.8 | 0.81 | 0.84 | 0.80 | 0.83 | 0.83 | 0.8 |
| **Ca** | 0.54 | 0.53 | 0.55 | 0.52 | 0.51 | 0.51 | 0. 52 | 0.52 | 0.52 |
| **Gross energy Kcal/ 100g[5]** | 450.27 | 449.31 | 450.04 | 447.39 | 448.02 | 450.16 | 451.61 | 447.08 | 448.9 |
| **P/E ratio (mg kcal$^{-1}$)[6]** | 66.74 | 68.44 | 67.22 | 68.06 | 67.63 | 66.82 | 67.09 | 67.66 | 67.9 |

1. Each Kg vitamin premix contained Vitamin A, 4.8 million IU, D3, 0.8 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
2. Each Kg mineral premix contained 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.
3. Axtra® PHY, sourced from a *Buttiauxella* species bacterium and is expressed in a *Trichoderma reesei* fungus **(Danisco Animal Nutrition)**.
4. Nitrogen free extract =100-(CP +EE +Ash+ CF)
5. GE= gross energy was calculated as 5.65, 9.45 and 4.12 Kcal/100gram of protein, lipid and carbohydrate, respectively after (NRC, 2011).
6. Protein/energy ratio (mg kcal$^{-1}$)

## Measurements of fish performance, feed utilization and survival rate (%)

Fish growth performance and feed utilization parameters were calculated as following equations:

1) Average weight gain (AWG, g / fish) = [final body weight-initial body weight]

2) Average daily gain, (ADG, g / fish / day) = [AWG/time (days)]
3) Specific growth rate (SGR, % / day) = [ln final weight - ln initial weight] × 100 /time (days)
4) Feed conversion ratio (FCR) = feed intake (g) /body weight gain (g)
5) Protein efficiency ratio (PER) = gain in weight (g)/ protein intake in feed (g)
6) Protein productive value (PPV% )= 100(protein gain in fish (g) /protein intake in diet (g))
7) Survival rate (SR %) = 100 x (total number of fish survived/total number of fish stocked).

**Chemical composition of fish and diet**

At the beginning and end of the experiment, 5 fish sample was taken randomly from each experimental group to determine chemical analysis of body composition. Experimental diets and fish carcass were conducted in order to determine the percentages of dry matter (DM %), crude protein (CP %), ether extract (EE %), crude fiber (CF %), and ash (Ash %) according to the **AOAC (2019)** method. Nitrogen free extract (NFE %) was calculated by differences, by deducting the sum of percentages of CP%, EE%, CF % and ash% from 100. Gross energy (kcal/100g DM,GE) contents of the experimental diets was calculated by using factors of 5.65, 9.45 and 4.12 kcal/100g of protein, lipid and carbohydrates, respectively **(NRC, 2011)**. Mineral was estimated by measuring phosphorus (P) and calcium (Ca) in experimental diets and whole body. All chemical analyses were carried in three replicates according to **(AOAC, 2019)**.

**Antibacterial Activity test:**

**Bacterial strains:** *Aeromonas hydrophila, Pseudomonas fluorescens, Enterococcus faecalis, Vibrio alginolyticus and Proteus mirabilis* were used in this study; these bacteria were isolated from Nile tilapia fish and identified phenotypically and genotypically according to **(Quinn *et al.*, 2011)**. All tested strains were kindly obtained by Prof. Mohamed Enany, professor of fish microbiology, bacteriology department, faculty of veterinary medicine, SCU.

**Assessment of antibacterial activity:**

Powder samples of phytase and citric acid and 4 antibiotic (nalidixic acid (30 µg), erythromycin (15 µg), amoxicillin (25µg) and cefadroxil (30µg)) were used because of its wide antibacterial spectrum and high potency, and most commonly used antibiotic against various diseases caused by Gram-negative and Gram-positive bacteria in fish farming) powder samples of phytase and citric acid were suspended in sterile distilled water with a powder/solvent ratio 1:1 (w/v) **(Bonilla and Sobral, 2017)**. Each tested

materials were mixed well by vortex, then putted in water bath at 40 C for 4 hours (modified) **(Choi *et al.*, 2016)**, then mixed well again by vortex (modified) **(Menezes-Blackburn *et al.*, 2015; Nassan *et al.*, 2015)** and the supernatant were collected. A prepared sterilized filter paper discs, 6 mm in diameter were thoroughly wet by immersing it in the obtained supernatant of tested samples. After incubation of the plates for 18 hours at 37°C the degree of sensitivity was calculated by measuring the clear zones of inhibition of growth produced by diffusion of the antibacterial agent from the discs into the surrounding medium. The results were interpretated according to **(CLSI, 2014).**

**Economic Evaluation:**

The cost of feed required to produce a unit of fish biomass was estimated using economic evaluation **(Samir *et al.*, 2017)**. The estimation was based on the local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: 25 L.E for fish meal; 6.5 L.E for soybean meal; 3.0 L.E for wheat bran; 3.0 L.E for Rice bran; 4.0 L.E for yellow corn; 20 L.E for sunflower oil; 30 L.E for Vit. and Min. mixture; 400 L.E. for phytase and 150 L.E. for citric acid.

**Feed cost of kg:** Calculated from the price of feed ingredient and the cost per kg gain (FCR × price of kg feed).

**Reduction % of feed cost of Kg gain** was calculated as a percentage from the highest value.

**Statistical analysis:**

Two ways ANOVA according to **Steel and Torrie (1981)** was used to compare between groups for each. Tukey test was used as a post hoc to compare mean differences at significant level 0.05 or 0.01 (P value). A Computer program software CoStat version 6.311 was used to analysis the data of experiments.

## Results and Discussion

Table 3 showed effect of supplementation different levels of phytase, citric acid and their interaction on growth performance, feed utilization parameters and survival rate of Nile tilapia (*O. niloticus*) fingerlings **for 60 days**. Data in the present study revealed that there was a significant difference ($P<0.05$) within citric acid concentration (0, 30 and 60 g/kg). The highest a significant difference ($p<0.05$) in final body weight, weight gain, weight gain%, specific growth rate, FI, FCR, FER**,** PER and PPV were recorded at fish fed D2 diet in comparison to the control and citric acid diets. This is in agreement with other studies on different species including *L. rohita* **(Bano and Afzal, 2017)**, hybrid grouper **(Anthonius *et al.*, 2018)**. This is may be due to lowered intestinal pH by citric acid, which

catalyzes the phytate-nutrient complex solubility and nutrients absorption from digestive tract resulting in improved growth performance of fish **(Cross *et al.*, 1990)**. In contrast, **Dai *et al.* (2018)** who found that there were no significant differences in specific growth rate, feed efficiency or feed intake among all dietary groups in turbot. This is may be due to factors such as species and physiological age of the experimental fish **(Gislason *et al.*, 1994)**, type and level of organic acids **(De Wet, 2005)**, diet composition **(Sarker *et al.*, 2007)** and culture conditions **(Ramli *et al.*, 2005)**.

Also, the results showed that there was a significant difference ($p$<0.05) within phytase concentration (0, 1000 and 2000 FTU/Kg). The group of fish fed on D4 had highest a significant difference ($p$<0.05) in growth performance and feed utilization in comparison to the control and phytase diets. This is in agreement with other studies on different species including tilapia **(Maas *et al.*, 2018 and Abo Norag *et al.*, 2018),** *L. rohita* **(Shah *et al.*, 2016 and Bano and Afzal, 2017)** and *Cirrhinus mrigala* **(Hussain *et al.*, 2017)**. The increased growth response may be due to increased availability of nutrients and minerals by enzymatic breakdown of phytate-nutrient complexes **(Shah *et al.*, 2016)**. In contrast, **Hu *et al*. (2016)** who found that there were no significant differences in WG and feed utilization between the experimental groups in tilapia. **Yigit *et al.* (2018)** reported that the addition of phytase to diets including soybean meal does not affect growth and FCR in rainbow trout. The differences between these studies may be due to phytase enzyme which depends on a variety of dietary factors such as the concentration and sources of phytate in the diet, concentration and sources of protein in the diet, types of feed ingredients used, methods used for feed drying, fish species, process of feed preparation, fish stomach pH and age of fish **(Dersjant-Li *et al.*, 2015 and Yigit *et al.*, 2018).**

The interaction was a significant ($p$<0.05) between citric acid and phytase on growth performance and feed utilization in comparison to the control and diets of interaction phytase and citric acid. The result revealed that fish fed D5 had the highest a significant different ($p < 0.05$) on growth performance and feed utilization in comparison to the control and diets of interaction phytase and citric acid. This is in agreement with other studies on different species including *L. rohita* **(Shah *et al.*, 2016 and Bano and Afzal, 2017).** This is may be due to role of citric acid in lowering the intestinal pH in the optimum conditions to the phytase, which led to

Table 3. Effect of supplementation different levels of phytase, citric acid and their interaction on growth Performance, feed utilization parameters and survival rate of Nile tilapia (*O. niloticus*) fingerlings for 60 days.

| Items | Experimental diets | | | | | | | | | Analysis of Variance (P value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D3 | D5 | D6 | D7 | D8 | D9 | Phytase (Phy) | Citric acid ( CA) | Phy×CA |
| **Initial weight (g/fish)** | 12.70 ± 0.28 | 12.69 ± 0.01 | 12.39 ± 0.28 | 12.38 ± 0.12 | 12.50 ± 0.20 | 12.76 ± 0.09 | 12.45 ± 0.30 | 12.17 ± 0.26 | 12.38 ± 0.39 | 0.19 | 0.86 | 0.94 |
| **Final weight (g/fish)** | 22.81 ± 0.40$^{f}$ | 28.09 ± 0.06$^{c}$ | 22.98 ± 0.01$^{f}$ | 30.73 ± 0.12$^{b}$ | 33.49 ± 0.31$^{a}$ | 23.81 ± 0.09$^{e}$ | 23.79 ± 0.12$^{e}$ | 25.80 ± 0.01$^{d}$ | 23.12 ± 0.42$^{ef}$ | 0.00** | 0.00** | 0.00** |
| **Weight gain (g/fish)** | 10.11 ± 0.12$^{g}$ | 15.40 ± 0.05$^{c}$ | 10.59 ± 0.28$^{fg}$ | 18.36 ± 0.00$^{b}$ | 20.99 ± 0.51$^{a}$ | 11.05 ± 0.01$^{ef}$ | 11.34 ± 0.18$^{e}$ | 13.63 ± 0.25$^{d}$ | 10.74 ± 0.03$^{efg}$ | 0.00** | 0.00** | 0.00** |
| **W. Gain %** | 79.61 ± 0.81$^{e}$ | 121.36 ± 0.34$^{c}$ | 85.47 ± 4.16$^{de}$ | 148.22 ± 1.46$^{b}$ | 167.92 ± 6.75$^{a}$ | 86.60 ± 0.72$^{de}$ | 91.08 ± 3.65$^{d}$ | 112.00 ± 4.39$^{c}$ | 86.75 ± 2.45$^{de}$ | 0.00** | 0.00** | 0.0001** |
| **Specific growth rate (SGR %g/day)** | 0.98 ± 0.01$^{e}$ | 1.32 ± 0.01$^{c}$ | 1.03 ± 0.04$^{de}$ | 1.52 ± 0.01$^{b}$ | 1.64 ± 0.04$^{a}$ | 1.04 ± 0.01$^{de}$ | 1.08 ± 0.03$^{d}$ | 1.25 ± 0.03$^{c}$ | 1.04 ± 0.02$^{de}$ | 0.00** | 0.00** | 0.0001** |
| **Feed intake (g/fish)** | 20.20 ± 0.18$^{f}$ | 26.03 ± 0.14$^{c}$ | 20.29 ± 0.05$^{f}$ | 30.09 ± 0.03$^{b}$ | 31.56 ± 0.15$^{a}$ | 21.88 ± 0.08$^{e}$ | 20.63 ± 0.08$^{f}$ | 23.55 ± 0.33$^{d}$ | 20.44 ± 0.17$^{f}$ | 0.00** | 0.00** | 0.00** |
| **Feed conversion ratio (FCR)** | 2.00 ± 0.01$^{a}$ | 1.69 ± 0.02$^{de}$ | 1.92 ± 0.06$^{b}$ | 1.64 ± 0.02$^{e}$ | 1.50 ± 0.03$^{f}$ | 1.98 ± 0.01$^{ab}$ | 1.82 ± 0.02$^{c}$ | 1.73 ± 0.01$^{d}$ | 1.90 ± 0.02$^{b}$ | 0.00** | 0.00** | 0.0048** |
| **Feed efficiency ratio (FER)** | 0.50± 0.01$^{f}$ | 0.59± 0.01$^{bc}$ | 0.52± 0.02$^{ef}$ | 0.61± 0.01$^{b}$ | 0.67± 0.01$^{a}$ | 0.51± 0.02$^{ef}$ | 0.55± 0.01$^{d}$ | 0.58± 0.01$^{c}$ | 0.53± 0.01$^{e}$ | 0.00** | 0.00** | 0.0049** |
| **Protein efficiency ratio (PER)** | 1.67 ± 0.01$^{e}$ | 1.92 ± 0.02$^{c}$ | 1.73 ± 0.05$^{e}$ | 2.00 ± 0.00$^{b}$ | 2.19 ± 0.04$^{a}$ | 1.68± 0.01$^{e}$ | 1.81 ± 0.02$^{d}$ | 1.91 ± 0.01$^{c}$ | 1.72 ± 0.02$^{e}$ | 0.00** | 0.00** | 0.0205* |
| **Protein productive value (PPV )** | 34.18 ± 0.18$^{d}$ | 40.37 ± 0.14$^{b}$ | 33.12 ± 0.05$^{d}$ | 38.44 ± 0.03$^{bc}$ | 45.09± 0.15$^{a}$ | 39.96± 0.08$^{b}$ | 45.13± 0.08$^{a}$ | 36.79 ± 0.33$^{c}$ | 31.46 ± 0.17$^{e}$ | 0.00** | 0.00** | 0.00** |
| **Survival rate (SR %)** | 90 ± 0.02 | 96 ± 0.03 | 93 ± 0.01 | 96 ± 0.02 | 97 ± 0.04 | 93 ± 0.01 | 93 ± 0.01 | 92 ± 0.02 | 92 ± 0.04 | 0.15 | 0.30 | 0.77 |

**Significant p-value ≤0.05, **highly significant p-value ≤0.01, using ANOVA test.*
*Different letters at the same raw mean significant different*

positive interaction among both supplements **(Shah *et al.*, 2016)**. In contrast, **Hussain *et al.* (2015)** who found that *Cirrhinus mrigala* fingerlings fed on diet having phytase 500 FTU $kg^{-1}$ combined with 5% citric acid enhance nutrient digestibility, growth rate. This is may be due to different fish species and experimental conditions.

There was no significant difference in survival rate (P>0.05) between phytase and citric acid either supplemented individually or mutually. This is in agreement with other studies on different species including tilapia **(Rachmawati *et al.*, 2018)** and hybrid grouper **(Anthonius *et al.*, 2018)**.

**Whole body chemical composition**

Table 4 showed effect of supplementation different levels of phytase, citric acid and their interaction on whole body composition (dry matter basis %) of Nile tilapia (*O.niloticus*) fingerlings for 60 days. Differences in the body compositions of fish may be due to age, size, water quality, season, geographic region, *etc*. **(Sener *et al.*, 2005)**; however, the main influential factors are diet quantity and quality **(Bell *et al.*, 2001)**. There was a significant difference ($p$<0.05) within citric acid concentration (0, 30 and 60 g citric acid /kg). Fish fed D2 diet showed the highest crude protein and ash body contents, while exhibited the lowest lipid content in comparison to citric acid diets. Also, It was observed that there were no significant differences (P>0.05) in body moisture content. This is in agreement with other studies on different species including *L. rohita* **(Shah *et al.*, 2016)** who reportd that citric acid supplementation improved all body composition and minerals but different with this study in moisture, and hybrid grouper **(Anthonius *et al.*, 2018)** who reportd that citric acid supplementation affected on body composition. In contrast, **Zhu *et al.* (2015)** who reportd that citric acid supplementation did not significantly affect body composition and mineral content of yellow catfish. **Dai *et al.* (2018)** who reported that turbot fed different diets showed similar whole-body proximate composition. This is may be due to fish species and experimental conditions.

There was a significant difference ($p$<0.05) within phytase concentration (0, 1000 and 2000 FTU/kg). Additionally, contents of body CP, ash, P and Ca increased, while total lipids decreased significantly as dietary D4. It was observed that there were no significant differences (P>0.05) in body moisture content. This is in agreement with other studies on different species including: Nile tilapia **(Nwanna and Olusola, 2014)** who reported that there were signifcant diference in min**e**rals composition P and Ca, shrimp **(Qiu and Davis, 2017)** found that a significant differences in CP and P but no significant in moisture of whole body.

**Table 4. Effect of supplementation different levels of phytase, citric acid and their interaction on whole body composition (% dry matter basis) of Nile tilapia (*O.niloticus*) fingerlings for 60 days.**

| Items | At the start | At the end | | | | | | | | | Analysis of Variance P value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | Phytase (Phy) | Citric acid ( CA) | Phy× CA |
| **Moisture (%)** | 74.93 ± 0.11 | 73.07 ± 0.12 | 73.45 ± 0.07 | 74.17± 0.01 | 75.22 ± 0.04 | 74.57 ± 0.03 | 71.14 ± 0.2 | 70.23 ± 0.05 | 74.49 ± 0.01 | 74.93 ± 0.02 | 0.20 | 0.32 | 0.21 |
| **Crude protein (%)** | 55.47 ± 0.05 | 59.30 ± 0.47$^{f}$ | 62.95 ± 0.15$^{c}$ | 60.05 ± 0.05$^{ef}$ | 64.45 ± 0.35$^{b}$ | 66.35 ± 0.15$^{a}$ | 60.58 ± 0.10$^{e}$ | 60.60 ± 0.30$^{e}$ | 61.80 ± 0.20$^{d}$ | 60.30 ± 0.20$^{e}$ | 0.00** | 0.00** | 0.00** |
| **Lipid (%)** | 22.73 ± 0.09 | 18.37 ± 0.27$^{a}$ | 10.93 ± 0.28$^{d}$ | 17.39 ± 0.26$^{a}$ | 8.37 ± 0.35$^{e}$ | 6.10 ± 0.21$^{f}$ | 14.58 ± 0.09$^{bc}$ | 14.70 ± 0.79$^{b}$ | 12.53 ± 0.33$^{c}$ | 15.07 ± 0.23$^{b}$ | 0.00** | 0.00** | 0.00** |
| **Ash** | 21.80 ± 0.04 | 22.33 ± 0.19$^{d}$ | 26.12 ± 0.13$^{b}$ | 22.56 ± 0.17$^{d}$ | 27.19 ± 0.70$^{a}$ | 27.55 ± 0.36$^{a}$ | 24.84 ± 0.13$^{c}$ | 24.70 ± 0.49$^{c}$ | 25.67 ± 0.04$^{bc}$ | 24.63 ± 0.03$^{c}$ | 0.00** | 0.00** | 0.0014 ** |
| **P** | 1.58 ± 0.07 | 1.34 ± 0.06$^{f}$ | 3.48 ± 0.03$^{b}$ | 1.38 ± 0.03$^{f}$ | 3.58 ± 0.02$^{b}$ | 3.96 ± 0.03$^{a}$ | 2.65 ± 0.02$^{d}$ | 2.71 ± 0.02$^{d}$ | 3.15 ± 0.02$^{c}$ | 1.53 ± 0.03$^{e}$ | 0.00** | 0.00** | 0.00** |
| **Ca** | 0.51 ± 0.03 | 0.15 ± 0.02$^{g}$ | 1.04 ± 0.02$^{c}$ | 0.21 ± 0.07$^{g}$ | 1.25 ± 0.07$^{b}$ | 1.38 ± 0.02$^{a}$ | 0.46 ± 0.02$^{f}$ | 0.59 ± 0.02$^{e}$ | 0.82 ± 0.02$^{d}$ | 0.36 ± 0.02$^{f}$ | 0.00** | 0.00** | 0.00** |

**Significant p-value ≤0.05, **highly significant p-value ≤0.01, using ANOVA test.*
*Different letters at the same raw mean significant different*

Also, **Shah *et al.* (2016)** who reportd that phytase supplementation improved body composition and minerals but different with this study in moisture in *Labeo rohita*. In contrast, **Abo Norag *et al.* (2018)** revealed that diets supplemented with phytase had no a significant effect on body moisture, CP, lipid, ash, Ca and P contents of Nile tilapia, **(Maas *et al.*, 2018)** revealed that Nile tilapia fed on phytase supplementation had significant difference in ash, lipid, P and Ca contents but difference with this study in moisture and CP contents **(Zhu *et al.*, 2015)** who reportd that phytase did not significantly affect body composition of yellow catfish but agreement with this study in mineral content as had significant.

There was a significant difference ($p<0.05$) in interaction between phytase and citric acid. Crude protein, ash, P and Ca of fish bodies increased and total lipid was decreased significantly ($p<0.05$) in fish fed dietary D5 in comparison to diets of interaction phytase and citric acid. It was observed that there were no significant differences ($P>0.05$) in body moisture content. This is in agreement with other studies on different species including *L. rohita* **(Shah *et al.*, 2016)** who reportd that phytase and citric acid supplementation improved body composition and minerals but different with this study in moisture of whole body composition**.** The improvement in nutrient, P and Ca in the fish body may be due to phytase and citric acid which can hydrolysis phytate by lowered pH and breakdown phytate complex.

**Antibacterial activity test**

Table 5 showed that there were different diameters of inhibition zone (mm) as antibacterial activity against different isolated bacteria from fish. Phytase alone was detected antibacterial activity against *Pseudomonas fluorescens*. It was recorded that mild inhibition zone diameter against *Pseudomonas fluorescens* only. In agreement, **Abo Norag *et al.* (2018)** who concluded that phytase supplementation up to 1000 units/kg diet significantly ($p\leq0.05$) improved the immune response and increased the survival rate of Nile tilapia fish after being challenged with *A. hydrophila*.

Citric acid was detected as the highest diameter of inhibition zone against tested bacteria compared to the rest treatments. The highest diameter of inhibition zone against *Pseudomonas fluorescens* then *Enterococcus faecalis* followed by *Vibrio alginolyticus, Aeromonas hydrophila* and *Protus mirabilis*. Studies were conducted to investigate the role of organic acids as antimicrobial effect on tilapia pathogen **(Abu Elala and Ragaa, 2015 and Koh *et al.*, 2016)** and shrimp **(He *et al.*, 2017)**. In contrast, **(Lim *et al.*, 2010)** showed no antibacterial effect on nile tilapia.

Then interaction between citric acid and phytase had effect on antibacterial activity. It recorded diameter of inhibition zone against tested bacteria. The highest diameter of inhibition zone against *Enterococcus faecalis* then *Aeromonas hydrophila* followed by *Pseudomonas fluorescens, Vibrio alginolyticus* and *Proteus mirabilis.* This may be due different feed additives, concentration or environmental conditions.

The results revealed that all tested antibiotics (Amoxicillin, Erythromycin and Cefadroxil) were detected resistance against *Aeromonas hydrophila, Pseudomonas fluorescens, Vibrio alginolyticus, Proteus mirabilis* and *Enterococcus faecalis.* While Nalidixic acid had antibacterial sensitivity to all tested bacteria but resistance against *Proteus mirabilis.*

**Table 5. Results of diameters inhibition zone (mm) of antibacterial activity against different isolated bacteria from fish.**

| Item | *Aeromonas hydrophila* | *Pseudomonas fluorescens* | *Vibrio alginolyticus* | *Proteus mirabilis* | *Entercoccus faecalis* |
|---|---|---|---|---|---|
| **Phytase** | R | 0.8 | R | R | R |
| **Citric acid** | 2.8 | 3.2 | 2.9 | 2.4 | 3 |
| **Citric acid and Phytase** | 1.5 | 1.4 | 1.4 | 0.8 | 1.9 |
| **Nalidixic acid** | 1.3 | 1.4 | 1.9 | R | 2.4 |
| **Amoxicillin** | R | R | R | R | R |
| **Erythromycin** | R | R | R | R | R |
| **Cefadroxil** | R | R | R | R | R |

R, Resistance

## Economic Evaluation

Table 6 showed that economic evaluation of experimental diets used in the study. Feed cost to produce one kg fish gain was reduced by inclusion of citric acid and phytase in single form. The reduction when fish fed D2 was 61.56%. The reduction when fish fed D7 was 41.35% but it was found that D4 was the best in term of growth. Feed cost to produce one kg fish gain was reduced by combination of CA and Phy. This reduction when fish fed D9 was 96.21% while, it was found that D5 (1000 FTU/Kg phytase+ 30g citric acid) was the best in term of growth performance. The costs of treatment from infections will decrease as this combination gave a healthy environment free from pathogenic bacteria.

**Table 6. Economic Evaluation of experimental diets used in the study.**

| Items | Experimental diets | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 |
| **Total** | 6.75 | 11.13 | 15.51 | 6.79 | 11.47 | 15.55 | 6.83 | 11.21 | 15.59 |
| **FCR** | 2.00 | 1.69 | 1.92 | 1.64 | 1.50 | 1.98 | 1.82 | 1.73 | 1.90 |
| **Feed cost/kg gain P.T** | 13.50 | 18.81 | 29.78 | 11.14 | 16.76 | 30.79 | 12.43 | 19.39 | 29.62 |
| **Reduction cost in kg gain (%)** | 43.85 | 61.09 | 96.78 | 36.18 | 54.43 | 100.00 | 40.37 | 62.97 | 96.20 |

## Conclusion

From the above mentioned, it can be concluded that; Phytase and citric acid positively affected the growth performance, feed utilization, whole body compassion and antibacterial activity against in Nile tilapia (*O. niloticus*) fingerlings. Moreover, both the supplements interacted significantly with each other for these parameters and that the fish fed on diet No. 5, containing 1000 FTU phytase / Kg with 30 g citric acid/ kg, gave the best results.

## Reference

**Abo Norag, M.A.; El-Shenawy, A.M.; Fadl, S.E.; Abdo, W.S.; Gad, D.M., Rashed, M.A. and Prince, A.M. (2018).** Effect of phytase enzyme on growth performance, serum biochemical alteration, immune response and gene expression in Nile tilapia. Fish Shellfish Immunology, 80**:** 97-108.

**Abu Elala, N.M. and Ragaa, N.M. (2015).** Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*. Journal of advanced research, 6**:** 621-629.

**Anthonius, C.; Seok Kian Yong, A. and Fui, C.F. (2018).** Supplementation of duckweed diet and citric acid on growth performance, feed utilization, digestibility and phosphorus utilization of TGGG hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) juvenile. Songklanakarin Journal of Science & Technology, 40**:** 1009-1016.

**Association of official Analytical chemists AOAC. (2019)**. Official Methods of Analysis of AOAC INTERNATIONAL, Arlington, Virginia, USA.

**Bano, N. and Afzal, M. (2017).** Synchronized effect of citric acid and phytase supplementation on growth performance and nutrient digestibility of *Labeo rohita*. Aquaculture Nutrition, 24**:** 786-792.

**Bell, J.G.; McEvoy, J.; Tocher, D.R.; McGhee, F.; Campbell, P.J. and Sargent, J.R. (2001).** Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (Salmo salar) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J Nutr, 131**:** 1535-1543.

**Bonilla, J. and Sobral, P.J.d.A. (2017).** Antioxidant and antimicrobial properties of ethanolic extracts of guarana, boldo, rosemary and cinnamon. Brazilian Journal of Food Technology, 20.

**Cao, L.; Wang, W.; Yang, C.; Yang, Y.; Diana, J.; Yakupitiyage, A.; Luo, Z. and Li, D. (2007).** Application of microbial phytase in fish feed. Enzyme and Microbial Technology, 40**:** 497-507.

**Choi, Y.J.; Jin, H.Y.; Yang, H. S.; Lee, S.C. and Huh, C.K. (2016).** Quality and storage characteristics of yogurt containing *Lacobacillus sakei* ALI033 and cinnamon ethanol extract. Journal of animal science and technology, 58**:** 16.

**Clinical and Laboratory Standards Institute CLSI. (2014).** Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement.

**Cross, H.; Debiec, H. and Peterlik, M. (1990).** Mechanism and regulation of intestinal phosphate absorption. Miner Electrolyte Metab, 16**:** 115-124.

**Dai, J.; Li, Y.; Yang, P.; Liu, Y.; Chen, Z.; Ou, W.; Ai, Q.; Zhang, W.; Zhang, Y. and Mai, K. (2018).** Citric acid as a functional supplement in diets for juvenile turbot, *Scophthalmus maximus* L.: Effects on phosphorus discharge, growth performance, and intestinal health. Aquaculture, 495**:** 643-653.

**Danisco Animal Nutrition** Axtra® PHY. http://www.danisco.com/animalnutrition.

**De Wet, L. (2005).** Organic acids as performance enhancers. Aqua Feeds: Formulation and Beyond, 2**:** 12-14.

**Dersjant-Li, Y.; Awati, A.; Schulze, H. and Partridge, G. (2015).** Phytase in non-ruminant animal nutrition: a critical review on phytase

activities in the gastrointestinal tract and influencing factors. Journal of the Science of Food and Agriculture, 95**:** 878-896.

**Gislason, G.; Olsen, R.E. and Ringø, E. (1994).** Lack of growth-stimulating effect of lactate on Atlantic Salmon, *Salmo salar* L. Aquaculture Research, 25**:** 861-862.

**He, W.; Rahimnejad, S.; Wang, L.; Song, K.; Lu, K. and Zhang, C. (2017).** Effects of organic acids and essential oils blend on growth, gut microbiota, immune response and disease resistance of Pacific white shrimp (*Litopenaeus vannamei*) against *Vibrio parahaemolyticus*. Fish Shellfish Immunology, 70**:** 164-173.

**Hu, J.; Ran, C.; He, S.; Cao, Y.; Yao, B.; Ye, Y.; Zhang, X. and Zhou, Z. (2016).** Dietary microbial phytase exerts mixed effects on the gut health of tilapia: a possible reason for the null effect on growth promotion. British Journal of Nutrition, 115**:** 1958-1966.

**Hussain, S.; Nisar, A.; Farhat, J.; Arshad, J.; Nosheen, A.; Majid, H.; Shahtaj, A.; Arsalan, M.; Danish, R. and Shahzad, M. (2015).** Effects of citric acid and phytase supplementation on nutrient digestibility and growth performance of *Cirrhinus mrigala* fingerlings fed on corn gluten (30%) meal based diets. International Journal of Biosciences (IJB), 6**:** 82-91.

**Hussain, S.M.; Hameed, T.; Afzal, M.; Javid, A.; Aslam, N.; Shah, S.Z.H.; Hussain, M. and Shahzad, M.M. (2017).** Growth Performance and Nutrient Digestibility of *Cirrhinus mrigala* Fingerlings Fed Phytase Supplemented Sunflower Meal Based Diet. Pakistan Journal of Zoology, 49**:** 1713-1724.

**Koh, C.B.; Romano, N.; Zahrah, A.S. and Ng, W.K. (2016).** Effects of a dietary organic acids blend and oxytetracycline on the growth, nutrient utilization and total cultivable gut microbiota of the red hybrid tilapia, O reochromis sp., and resistance to S treptococcus agalactiae. Aquaculture Research, 47**:** 357-369.

**Lim, C.; Klesius, P.H. and Lückstädt, C. (2010).** Effects of dietary levels of potassium diformate on growth, feed utilization and resistance to *Streptococcus iniae* of Nile tilapia, *Oreochromis niloticus*. Proceedings of the fourteenth International Symposium on Fish Nutrition and Feeding.

**Maas, R.M.; Verdegem, M.C.J.; Dersjant-Li, Y. and Schrama, J.W. (2018).** The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. Aquaculture, 487**:** 7-14.

**Menezes-Blackburn, D.; Gabler, S. and Greiner, R. (2015).** Performance of seven commercial phytases in an in vitro simulation of poultry digestive tract. Journal of Agricultural and Food Chemistry, 63**:** 6142-6149.

**Nassan, M.; Mohamed, E.; Abdelhafez, S. and Ismail, T. (2015).** Effect of clove and cinnamon extracts on experimental model of acute hematogenous pyelonephritis in albino rats: Immunopathological and antimicrobial study. International journal of immunopathology and pharmacology, 28**:** 60-68.

**Ng, W.K. and Romano, N. (2013).** A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. Reviews in Aquaculture, 5**:** 220-254.

**National Research Council NRC. (٢٠١١).** Nutrient Requirement of Fish. , National Academic Press, , Washington, DC.

**Nwanna, L. and Olusola, S. (2014).** Effect of supplemental phytase on phosphorus digestibility and mineral composition in Nile tilapia (*Oreochromis niloticus*). International Journal of Aquaculture, 4**:** 89-95.

**Qiu, X. and Davis, D. (2017).** Effects of dietary phytase supplementation on growth performance and apparent digestibility coefficients of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture Nutrition, 23**:** 942-951.

**Quinn, P.J.; Markey, B.K.; Leonard, F.C.; Hartigan, P.; Fanning, S. and Fitzpatrick, E.i. (2011).** Veterinary microbiology and microbial disease, John Wiley & Sons.

**Rachmawati, D.; Samidjan, I. and Elfitasari, T. (2018).** Effect of The Phytase Enzyme Addition in The Artificial Feed on Digestibility of Feed, Feed Conversion Ratio and Growth of Gift Tilapia Saline Fish (*Oreochromis niloticus*) Nursery Stadia I. Pages 012009. IOP Conference Series: Earth and Environmental Science. IOP Publishing.

**Rachmawatia, D., Samidjana, I. and Melb, M. (2017).** Effect of Phytase on Growth Performance, Diet Utilization Efficiency and Nutrient Digestibility in Fingerlings of Chanos chanos (Forsskal 1775). Philippine Journal of Science, 146, 237-245.

**Ramli, N.; Heindl, U. and Sunanto, S. (2005).** Effect of potassium-diformate on growth performance of tilapia challenged with *Vibrio anguillarum*. World Aquaculture Society Conference. Abstract, CD-Rom, Bali, Indonesia.

**Samir, F.; Abd El-Naby, A.S.; Khattaby, A.E.R. and Awad, S.M.M. (2017).** Improvement of Growth Rate, Feed Utilization, Body Composition and Immune Response of Nile Tilapia (*Oreochromis niloticus*) By Supplementation of Nucleotide in Diets. Pages 62-88. 1st International Conference (Central Laboratory For Aquaculture Research In Cooperation With Worldfish).

**Sarker, M.S.A.; Satoh, S. and Kiron, V. (2007).** Inclusion of citric acid and/or amino acid-chelated trace elements in alternate plant protein source diets affects growth and excretion of nitrogen and phosphorus in red sea bream *Pagrus major*. Aquaculture, 262**:** 436-443.

**Sener, E.; Yildiz, M. and Savaş, E. (2005).** Effects of Dietary Lipids on Growth and Fatty Acid Composition in Russian Sturgeon (*Acipenser gueldenstaedtii*) Juveniles. Turk J Vet Anim Sci, 29**:** 1101-1107.

**Shafique, L.; Afzal, M.; Shah, S.Z.H.; Fatima, M.; Naz, H. and Saddique, Q. (2018).** Acidified Diet Increases the Trace Mineral Content in Whole Body Fish *Labeo rohita* Fingerlings. Punjab University Journal of Zoology, 33**:** 103-106.

**Shah, S.Z.H.; Afzal, M.; Akmal, A.; Fatima, M. and Hussain, S.M. (2016).** Effect of citric acid and phytase on growth performance and mineralization of *Labeo rohita* juveniles fed soybean meal based diet. Int. J. Agric. Biol, 18**:** 111-116.

**Steel, R.G.D. and Torrie. J. H. (1981).** Principles and procedures of Statistics. A Biometrical approach. Second ed. McGraw-Hillpp,167-173.

**Yigit, N.O.; Bahadir Koca, S.; Didinen, B.I. and Diler, I. (2018).** Effect of protease and phytase supplementation on growth performance and nutrient digestibility of rainbow trout (*Oncorhynchus mykiss*, Walbaum) fed soybean meal-based diets. Journal of Applied Animal Research, 46**:** 29-32.

**Zhu, Y.; Ding, Q.; Chan, J.; Chen, P. and Wang, C. (2015).** The effects of concurrent supplementation of dietary phytase, citric acid and vitamin D3 on growth and mineral utilization in juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquaculture, 436**:** 143-150.

# تأثير انزيم الفيتيز وحمض الستريك على أداء النمو وكفاءة الأستفادة من الغذاء و نشاطهما ضد البكتريا الممرضة فى أصبعيات البلطى النيلي

**نهاد محمود[١]، عبد الحميد عيد[١]، بديعة عبد الفتاح[١]، على وهدان[٢]، محمد السيد عنانى[٢] و اسماء سمير عبد النبي[٣]**

[١] كلية زراعة – جامعة قناة السويس- الاسماعيلية - مصر
[٢] قسم البكتريولوجيا والمناعة والفطريات – كلية الطب البيطرى – جامعة قناة السويس- الاسماعيلية- مصر
[٣] قسم بحوث تغذيه الاسماك- المعمل المركزى لبحوث الثروة السمكية – العباسة- ابو حماد - الشرقية - مصر

## الملخص العربي

تهدف تلك التجربة الى دراسة تأثير انزيم الفيتيز و حمض الستريك والتفاعل بينهما على اداء النمو، وكفاءة الأستفادة من الغذاء ومحتوى جسم الأسماك والتقييم الاقتصادى و نشاطهما ضد البكتريا الممرضة فى أصبعيات البلطى النيلي. تم اعداد تسعة علائق معملية متوازنة في البروتين ٣٠٪ ومتوزنة في الطاقة ٤٥٠,٢٧ كيلوكالورى/ ١٠٠جم. تم اضافة ثلاث مستويات مختلفة من انزيم الفيتيز (٠، ١٠٠٠، ٢٠٠٠ FTU/كجم عليقة) بينما تم اضافة ثلاث مستويات مختلفة من حمض الستريك (٠، ٣٠، ٦٠ جم/كجم عليقة ). وتم تخزين ٦٧٥ أصبعية من أسماك البلطى النيلى عشوائيا فى ٢٧ فايبر تانك على شكل حرف v سعة التانك ١٢٠ لتر وكانت لكل معاملة مستخدمة فى تلك الدراسة ثلاث مكرارات. تم وضع فى كل تانك ٢٥ أصبعية بمتوسط وزن أبتدائى ±12.47 0.3 جم / سمكة. تمت اقلمة الاسماك على الظروف المعملية لمدة اسبوعين قبل بداية التجربة. تم تنظيم فترات الاضاءة ١٢ساعة اضاءة /١٢ ساعة اظلام. تمت تغذية الاسماك يدويا مرتين يوميا (٦ أيام فى الأسبوع ) حتى الشبع لمدة ٦٠ يوم. واشارت النتائج الى ان مجموعة أصبعيات أسماك البلطى النيلي التى غذيت على العليقة رقم ٥ والمحتوية على ١٠٠٠ وحدة من أنزيم الفيتيز مع ٣٠ جرام حمض السترك أعطت افضل النتائج لإداء النمو وكفاءة الأستفادة من الغذاء مقارنة بالعليقة القياسية ( بدون أضافة ). بينما أظهر النشاط ضد البكتريا أن حمض الستريك ومجموعة حمض الستريك مع انزيم الفيتيز لهما نشاط عالى و تثبيط واسع ضد البكتريا المختبرة والمعزولة من أسماك البلطى النيلي المصابة بالبكتريا مقارنة بالمضادات الحيوية المختلفة. اظهر الفيتيز وحمض الستريك تأثيرا ايجابيا على اداء النمو، كفاءة الاستفادة من الغذاء، محتوى جسم الاسماك و النشاط ضد البكتيريا الممرضة ضد اصبعيات البلطى النيلي. كما اظهر كل من المكملات تفاعل تاثيرا معنويا بينهما البعض على تلك القياسات وحيث كانت افضل نتيجة لمجموعة الاسماك التى غذيت على العليقة رقم ٥ المحتوية على ١٠٠٠ وحدة من أنزيم الفيتيز مع ٣٠ جرام حمض الستريك.