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Effect of different levels of citric acid as supplementation on growth performance, feed utilization, body composition, water quality, and blood profile of Monosex Male Nile Tilapia (*Oreocheromis niloticus*) fingerlings

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ABSTRACT

This study was conducted to evaluate the use of a dietary citric acid as organic acid supplementation on growth performance, feed utilization, hematology and immune response of Nile tilapia, *O. niloticus.* A total of 720 apparently healthy *O. niloticus* fingerlings with an averages of body weight and length 5.6 ± 0.14 g and 7.0 ± 0.11 (mean ± SE) were randomly divided into three equal groups (G1, G2 and G3). G1 was fed a basal diet (without citric acid supplementation), while G2 and G3 were fed a dietary citric acid supplementation mixture in 0.5 and1 g/kg diet, respectively in triplicate for 90 days (from 1/8/2017 to 30/10/2017). At the end of period (90 days), groups G2 and G 3 had significant ($P > 0.05$) in all the parameters analysed (water quality parameters, growth performance, feed utilization, hematology and immune response of male Nile tilapia (*O. niloticus*) fingerlings compared with the control diet group(G1). Blood parameters were significantly ($P < 0.05$) lower in the control diet than the other experimental diets, which did not significantly differ from each other ($P > 0.05$). Fish fed diet G3 (supplemented with 1.0 % citric acid / kg diet) showed the higher serum total protein content, while the control group showed the lower serum haematological parameters compared with the other groups ($P \leq 0.05$). The average value of (ALT), and (AST) were significant decreased ($P< 0.05$) compared with control and other diet the addition *of* citric acid. The present observations suggest that, the growth, feed utilization, and health status of *O. niloticus* fingerlings can be enhanced with a supplementation with a dietary citric acid as organic acid supplementation improves growth performance, feed utilization, Proximate composition of body muscle, hematology and immune respons of Nile tilapia, *O. niloticus* fingerlings.

INTRODUCTION

Over the last 20 years, aquaculture is growing more rapidly than all other animal food production sectors (FAO, 2016). The expansion of aquaculture production has been accompanied by the rapid growth of aquafeed production. Aquaculture is the fastest-growing animal food-producing sector in the world, whose potential to meet the challenges of food security, and generate jobs and economic gains have been clearly demonstrated (FAO, 2016). Therefore, it is essential to find ways to develop the activity in a sustainable way, seeking to increase production without compromising natural resources, based on a vision of food and nutritional security (Hlpe, 2014). Nutrition is one of the most important factors influencing the ability of cultured tilapia to exhibit its genetic potential for growth and it is greatly influenced

by factors such as the behavior of fish, stocking density, quality of feed, daily ration size, feed frequency and others. In addition to the above factors, the use of feed containing functional feed additives in tilapia nutrition improve feed conversion ratio, promote the growth and health of tilapia, improve their immune systems, and induce physiological benefits beyond traditional feeds (Souza, 2010). Feed additives are supplemented in small amounts (alone or in combination) for a specific purpose, such as to improve the quality of fish as a final product, to preserve the physical and chemical quality of the diet or to maintain the quality of the aquatic environment (Bai *et al*. 2015). Additives are used in fish feed to preserve the nutritional characteristics of a diet or feed ingredients prior to feeding (e.g. antioxidant and mold inhibitors (Bai *et al.* 2015) enhance ingredient dispersion or feed pelleting (e.g. emulsifiers, stabilizers and binders Pigott and Tucker (2003) facilitate feed ingestion and consumer acceptance of the product (e.g. feed stimulants or attractants) He *et al.* (2012) and growth promote (e.g. growth promoters, including antibiotics, probiotics and hormones (El-Sayed *et al.* 2012). Organic acids are organic carboxylic compounds of general structural formula R-COOH whose acidity is associated with their carboxyl group (-COOH). Hey is weak acids because they partially dissociate in water to form a hydrogen ion (H^+) and a carboxylate ion ($-COO^-$) (e.g. acetic) (Lim *et al.* 2015). Dietary acidification by the addition of organic acids has been widely used in animal nutrition and organic acids have become a promising feed additive to improve gut health and performance (Hassaan *et al.* 2015). Organic acids also used in fish feed to reduce the potential threat of microbial contamination including pathogenic bacteria and molds or fungi (due to Aspergillus, *Penicillium*, and *Fusarium*) that may grow during feed storage (Lim *et al.* 2015). He most commonly used organic acids as feed additive includes: (1) individual or combinations of organic acids such as propionic, sorbic, benzoic, butyric acids, malic acid, lactic acids, and acetic acids, and (2) salts of organic acids such as calcium propionate, potassium sorbate, and sodium benzoate (Bai *et al.* 2015 and Lim *et al.* 2015). According to (Abu-Elala and Ragaa 2015) reported that *O. niloticus* fed on 0.2% and 0.3% organic acid, potassium diformate (KDF) exhibited significant improvements in their feed intake, live weight gain, specific growth rate, feed conversion ratio (FCR) and protein efficiency ratio compared with control. Because the low molecular weight organic acids can diffuse across the cell membrane of gram-negative bacteria, acidification of their metabolism can lead to bacterial cell death. Various concentrations of organic acids such as propionic acid and acetic acid, have been determined to have effects on the feeding behavior of *Oreochromis niloticus*. The supplementation of propionic acid at 10^{-4}–10^{-6} M can stimulate feeding. However, dietary propionic acid at 10^{-3} M may suppress feeding. In addition, past research has also found that dietary supplementation of acetic acid at 10^{-5} M had no effect on fish feeding, and water quality (Lim *et al.* 2015). In general, dietary organic acid supplementations are an undigested feed ingredient (Gibsen and Roberfroid 1995) that benefits fish by selectively stimulating growth (Talpur *et al.* 2014).Therefore the present research aimed to study the possibility of feeding Nile Tilapia diets containing graded lever of citric acid (0.5 and1g/kg).

MATERIALS AND METHODS

Experimental Husbandry farming

The present experiment was conducted at the indoor experimental fish culture concrete tanks with closed a water recirculation system, belonging to the fish production section, Animal production department, faculty of agriculture, Al-Azhar University, Cairo, Egypt.

Experimental Aquaculture Units

The experimental rearing system consisted of a series of 9 rectangular concrete tanks represent three groups (in triplicate per group) with the same average water volume of four m^3 (1mx 4m x1 m) in a closed recycling water system. The water supply of these tanks is the drinking tap water which derived the mechanical filter reservoir via a pump to another two fiberglass tanks of 5 m3 capacity. A series of concrete tanks connected together with a tap water supply as well as a drainage system and connected with a mechanical filter. All experimental tanks were supplied with air through an aeration system, which connected with an oil-free air compressor (30 hv). Tap water has been stored for two days in two fiberglass tanks of 5 m3 capacity for dechlorination and for filling the experimental tanks and replacing the changed water at (100 % of tank water twice/week).

Experimental Fish and maintenance:

The fish used in this study were purchased from a private tilapia hatchery in. Kafr El-Sheikh Governorate, Egypt. The experimental fish were transported in the early morning using a special fish transport car with aeration facilities. They were acclimated to the experimental conditions and hand-fed commercial diet twice daily to apparent satiation for 15 days.

After acclimation, a total number of 720 apparently healthy male Nile tilapia (*Oreocheromis niloticus*) fingerlings were randomly distributed into 9 tanks as triplicates in which 80 fingerlings were stocked in each tank ($4m^3$)

Fish were hand-fed one of three experimental diets to apparent satiation, twice a day, 6 days a week for 12 weeks. The uneaten feed per tank was siphoned out, dried, and weighed. The amount of feed consumed was then accurately quantified for each dietary group.

Experimental Diets

The control diet contained no feed supplements. All the prepared diets contained 30% crude protein and 5% crude lipid. The feed ingredients and proximate composition of the diets (Tables 1 and 2) were analyzed using the Association of Official Analytical Chemists (AOAC) methodologies (AOAC 2000).Dry matter (DM %) composition and chemical analysis (crude protein (CP, %) , ether extract (EE, %) , ash (%) , crude fiber (CF %) , Nitrogen- free extract (NFE, %) and gross energy (GE(Kcal/kg DM) of such main ingredients are presented in Table (1).While, the experimental diets composed mainly of fish meal, Gluten, soybean meal, yellow corn, wheat bran, Soybean oil, vitamins, and minerals mixed and Citric acid, and calculated analysis of basal diet are shown in Table (2).

The dietary experimental ingredients were finely ground, weighed according to their percentage and then mixed together. Some warm water was added to each diet to be easily pelleted by pressing through 2mm die by a meat mincer machine. The pellets were dried in a drying oven at 60°C for 24 hours and crushed to adjust the diameter of pellets according to fish size and then stored at -4°C to avoid oxidation and rancidity.

Table 1: Chemical analyses of the feed ingredients used in the experimental diets.

Item / Ingredients	DM %	CP %	EE %	Ash %	CF %	*NFE %	Total	**GE (Kcal/kg DM)
Glutein	92.66	69.50	2.20	1.9	2.00	24.4	100.00	513.03
Fish meal	93.58	60.52	8.43	25.5	0.60	4.95	100.00	441.25
Wheat bran	91.22	16.57	1.46	4.1	9.90	67.79	100.00	385.84
Soybean meal	92.50	48.10	1.23	6.3	7.30	37.07	100.00	435.24
Yellow corn	90.55	7.59	1.83	0.8	2.30	87.48	100.00	419.61

* Calculated by differences [Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)]

** Gross energy value was calculated from their chemical composition, Estimated according to NRC (1993). as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

Accordingly, this experimental diet (isonitrogenous , 30% CP and isocaloric , 19.5 MJ kg^{-1}, GE/kg.) contained three treatments groups including the control group which received the basal diet free of dietary citric acid supplementation as follow :

D1(group 1)=control = group received the basal diet free of dietary citric acid supplementation

D2(group 2) =group received the basal diet + 0.5 g /kg citric acid supplementation

D3(group 3) = group received the basal diet + 1.0 g / kg citric acid supplementation

Feeding technique

Diets were fed to each group of fish during the experimental period in the form of dried pellets suiTable to fish size. The feeding level was 3% of the total biomass of the fish /day. Fish were fed 6 days/week and the amount of feed was divided into two equal portions at 9 a.m and 2 p.m. Every seven days, the fish in each tank were weighed and the amount of feed was corrected according to the new fish biomass throughout the experimental period The actual experimental feeding trials curated for a period of 3 months (90 days).

Table 2: Ingredients, chemical composition and calculated analysis of the formulated and basal diet used in the experimental diets (on DM basis)

Ingredients %	Experimental diets		
	Diet (1) (group1) Control	Diet (2) (group2) 0.5 **g** Citric acid	Diet (3) (group3)1 **g** Citric acid
Fish meal	5	5	5
Gluten	15	15	15
Soybean meal	25	25	25
Yellow corn	38	37.5	37
Wheat bran	12	12	12
Soybean oil	4	4	4
Vit. & mineral[1]	1	1	1
Citric acid	0	0.5	1
Total	100	100	100
Chemical analysis (determined on dry matter basis)			
Crude Protein (%)	30.15	30.17	30.14
Crude Lipids (%)	5.54	5.35	5.26
Ash (%)	5.11	5.32	5.32
NFE[2] (%)	59.20	59.16	59.28
GE (MJ kg^{-1})[3]	19.49	19.53	19.50

[1]Vitamin and mineral mix (mg or g / Kg diet): MnSO4, 40 mg; MgO, 10 mg; K2SO4, 40 mg; ZnCO3, 60 mg; KI, 0.4 mg; CuSO4, 12 mg; Ferric citrate, 250 mg; Na2SeO3, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU; α-tocopherol acetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg; ;nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; ascorbic acid, 500 mg.

[2]NFE (Nitrogen free extract)=100-(crude protein + lipid + ash).

[3]Calculated using gross caloric values of 23.63, 39.52 and 17.15 kJ/g for protein, fat and carbohydrate, respectively according to Brett (1973)

Growth performance and feed utilization parameters.

As mentioned before, fish were weighed at weekly intervals (to minimize the effect of handling) as reported by El-Banna (1991). Each fish was individually removed with a small soft towel, dried using tissues, and the weight and length were recorded; fish were subsequently released to their respective tanks, which were filled with clean new water.

- Final weight gain (g) = final wt. (g) - initial wt. (g)

- **Average daily gain (**ADG) = (Final body weight (g)(W_1) - Initial body weight (g) (W_0) /T (Experimental period (days)

. Specific growth rate (SGR % /day) = (ln final wt.(W1) - ln initial wt (W_0) / period (days) × 100, Where : Ln = Natural logarithm

- Condition factor (K):

$$K = \frac{\text{Final fish weight [g]}}{L^3\,(\text{cm})} \times 100$$

Survival rate (SR %) =(Final number of fish / Initial number of fish) × 100

Moreover, the food intake (Feed consumed) (FI) ,food conversion ratio (FCR) , Feed efficiency (FE %) , Protein efficiency ratio (PER %) , Protein productive value (PPV %) and Energy retention (ER %).

Survival rate=No. survive fish / Total number stock×100

Feed intake **(**Feed consumed **) (FI):**

This is obtained by adding daily mean feed intake (DFI) of fish under each treatment for the experiment period.

Feed consumed (total food intake) (g/fish) = total spread feed - extra remained feed-in tank.

Feed Conversion Ratio (FCR):

This is calculated by dividing the total amount of feed given (feed intake) TFI by the mean weight gain (MWG). FCR is the proportion of dry feed per unit live weight gain of fish.

(FCR) = feed intake (g) (feed given per fish)/(weight gain per fish) x 100

Feed efficiency (FE %) = [Weight gain (g) / Feed intake (g)]**.**

(PER %) = (wet weight gain per fish)/(total protein intake per fish) × 100.

PPV(%)= Retained protein (g) / protein intake (g) ×100.

Chemical analysis of feed and fish body:

Diet samples were oven-dried 105ºC for 24 h, ground, and stored at −20ºC for subsequent analysis. Fish and diet samples representative samples were chemically analyzed for proximate composition according to the AOAC (2000).

Water quality parameters :

Water temperature, dissolved oxygen and pH were analyzed and recorded on the dike of the tank in triplicate. Water temperature, pH, salinity and dissolved oxygen (DO-mg/l) were measured twice daily (around 09:00 am and 14:00 pm); although these four measurements did not change significantly (because of the indoor, closed, non-circulating, continuously aerated water environment), it was important to document the cleanliness of the aquaculture tank .Water temperature was measured by thermometer model Thermo-Orion, pH was measured with a porTable pH meter and Dissolved oxygen (DO, mg/L) was measured using a porTable Microprocessor auto cal. DO meter (Model HI 9143, Sensitivity±0.01 mg/L, HANNA Instruments, Portugal).

Weekly measurements were taken of unionized ammonia nitrogen (NH_3-N), Nitrate (NO_3-N) and nitrites-nitrogen (NO_2-N) (mg/l) concentrations were measured

at intervals of 7 days.The concentration of unionized ammonia nitrogen (NH_3-N) was measured by using pH meter (Unicam, 9450 pH Meter, Sensitivity± 0.001/0.01, Cambridge, UK) and ammonia electrode (Model IS 570-NH3, Sensitivity 57±-2m V, Philips) . Nitrate (NO_3-N) (mg/l) and Nitrite nitrogen (NO_2-N) were determined using sulphanilamide in an acid solution (titration methods explained by Boyd and Tucker,1998).

Serum collecting, hematological and biochemical blood examination:

For the hematological analysis, at the end of the 90 days of the feeding period, fish were collected from the culture tanks, after final weighing, blood samples were taken from ten fish samples from each of the treatment and control groups tanks were randomly collected using hand net. The captured fish were degutted and cleaned with tap water and blood samples were collected from the caudal vein of fish from treatments. Blood was drawn into haematocrit tube from this sample and divided into two portions. The first portion was collected with anticoagulant 10% ethylene diamine tetraacetate (EDTA) to determine the hemoglobin (Hb) and Serum total protein levels (TP).

The haemoglobin (HB g\dl), the cyamethahaemoglobin method was used. 0.02cm³ of blood was placed in 4cm³ of Drabkins reagent in a test tube and mixed. After 30minutes, the tropical density was read calorimetrically at 450. The values of haemoglobin were determined according to the standard methods as described by Rawling (2009). The second portion of the blood samples was allowed to clot overnight at 4°C and then was centrifuged at3,000 rpm for 10 min. The non hemolysed serum was collected and stored at−20°C until use.

Haematological parameters:

The following parameters were used to assess the effects of dietary treatments on the haematological profile of *O. niloticus* at the end of the feeding trials.The total count of red blood cells leukocytes(RBC; 10^6 /ml) , white blood cell count (Leucocytes , WBC; 10^3/ml) was carried out by the indirect method described by Martins *et al.* (2008). For the differential count of defense cells (leukocytes), blood smears were stained according to the method described by Svesbodora, Fravda, and Palakova (1991), where 100 cells were counted in each slide and the result expressed in% of each cell type (neutrophil, lymphocyte, monocyte and PLAT (u\l) cell). Then the absolute number of each was calculated with respect to the total number of leukocytes observed in the Newbauer chamber.

Differential leukocyte counts (neutrophils, lymphocytes and monocytes) were conducted on May-Grunwald- Giemsa stained blood smears.

Hematimetric variables (PVC) was read by a microhaematocrit reader and expressed as a volume of erythrocyte per 100 cm³ (Rawling, 2009). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) were determined mathematically calculated according to the method of Stoskopf (1993) and Meyer *et al*, (1992) relationship, where: MCV (fL) = [(hematocrit No. × 10)/No. of erythrocytes]; MCH (pg cell-1) = [(Hemoglobin x 10) / No. of erythrocytes]; MCHC (g dL-1) = [(Hemoglobin x 100)/Hematocrit]. Total serum protein (TP) (g\dl) was determined according to Doumas, Bayse, Carter, Peters, and Schaffer (1981).

Biochemical blood indices :

The immune response of fish as affected by treatments (Levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) activities, were estimated according to the method described by Reitman and Frankel (1957).

Statistical analysis:

All the results of the present study were expressed as means ± standard error (SE) and data were analyzed using (SPSS, 1997). One-way analysis of variance (one-way ANOVA) was used to determine whether there was significant variation among the treatments. When overall differences were found, differences between means were tested by Duncan multiple range test.

RESULTS AND DISCUSSION

Water quality parameters:

From Table (3), the water quality parameters during the study showed that the diets containing different doses of dietary citric acid as a growth promoter supplementation of sex-reversed all-male Nile tilapia (*O. niloticus*) fingerlings during the experimental period (90 days) had no effects on the water quality culture. All tested water-quality criteria were suiTable and within the accepTable limits for rearing the Nile tilapia (*O. niloticus*) fingerlings (Boyd 1990) and Asriqah L *et al.* (2018).

These findings are consistent with a past study by stating that water quality parameters such as temperature, dissolved oxygen and pH measured in a similar current experimental are all within the accepted range for the culture of finfishes in tropical regions, as recommended by National Research Council (USA)(NRC, 1993).

Table 3: Effect of diets containing different doses of dietary citric acid as a growth promoter supplementation on water quality parameters of Nile tilapia (*O. niloticus*) fingerlings during the experimental period (90 days ,three replicates ,range and mean range ± standard errors).

Water quality parameters	Experimental diets		
	Diet(1)Control	Diet (2) 0.5 **gm** Citric acid	Diet (3)1 **gm** Citric acid
Water Temperature (°C)	(23.5-30.5) 27.0±0.34a	(24.1 - 30.5) 27.3 ±0.21a	(24 - 30.9) 27.45±0.22a
Dissolved Oxygen (O_2 - mg/l)	6.6 -7.3 6.9±0.21a	7.1 -8.1 7.6 ±0.23a	6.2 -8.2 7.2 ±0.11a
pH (pH unit)	8.2 - 8.3 8.5±0.2a	7.83 - 8.4 8.2 ±0.3a	8.19 - 8.3 8.3±0.1a
un-ionized ammonia ((NH_3- N mg/L)	0.03-0.4 0.12 ±0.03a	0.06-0.4 0.14 ±0.05a	0.03-0.23 0.13 ±0.02a
Nitrite (NO_2-N mg/l)	0.06-0.11 0.055±0.02a	0.033-0.66 0.047 ±0.01a	0-0.1 0.05 ±0.02a

Mean ± SE followed by the same superscript letter (a) indicated not significantly different at $P < 0.05$.

Water quality refers to that with adequate oxygen, proper temperature, transparency, limited levels of metabolites, and optimum levels of other environmental factors affecting fish culture. This information would enable the farmers to take better care of their fish ponds by frequently monitoring the conditions of the ponds, fish behavior, and water color for any abnormal changes.

Effects on Growth performances indices:

At the end of the experiment, the inclusion of dietary citric acid as a growth promoter supplementation (Table 4) resulted in a significantly (p<0.05) improved the growth performance of sex-reversed all-male Nile tilapia (*O. niloticus*) fingerlings during the experimental period compared with the control group. The higher final body weight (BW), final body length (BL), weight gain (WG), and specific growth rate (SGR) were found in fish fed the diet supplemented with 1% citric acid

supplementation (D3), while the control group (D1) showed the lower values of these response parameters ($P < 0.05$).

Table 4: Effect of diets containing different doses of dietary citric acid as a growth promoter supplementation on growth performance parameters of Nile tilapia (*O. niloticus*) fingerlings.

parameters	Experimental diets		
	Diet (1) Control	Diet (2)0.5 **gm** Citric acid	Diet (3)1 **gm** Citric acid
Average initial weight (g/fish)	**5.6**	**5.5**	**5.3**
Average final weight (g/fish)	**64.95±0.15^{c}**	**66.90±0.20^{b}**	**67.10±0.10^{a}**
Average final length (cm)	**15.25±0.15^{c}**	**15.80±0.20^{b}**	**15.90±0.10^{a}**
Average Total weight gain (g/fish)	**59.95±0.15^{c}**	**61.90±0.20^{b}**	**62.10±0.10^{a}**
Average daily gain(g/fish/day)	**0.67±0.01^{b}**	**0.69±0.02^{a}**	**0.69±0.01^{a}**
Specific growth rate (SGR % day -1)	**2.39±0.09^{a}**	**2.42±0.07^{a}**	**2.42±0.1^{a}**
Condition factor (K)	**1.73±0.05^{c}**	**1.80±0.07^{b}**	**1.87±0.03^{a}**
No. of fish at start.	**240**	**240**	**240**
Survival rate (SR %)	**99**	**100**	**100**

a,b,c…. etc: means ± S.E. within the same row with different superscripts are significantly different ($P \leq 0.05$).

From Table 4, there were significant differences ($P < 0.05$) on growth performance as influenced by feeding dietary citric acid supplementation (Table 4). Fish fed diet 1(control) showed the lowest final body weight (64.95 g/fish), final fish length (15.25 cm), total gain in weight (59.95g/fish), average daily gain in weight (0.67%) and specific growth rate (2.39 % day-1), condition factor (1.73) and survival ratio (99 %) compared with others. While fish fed diet 3 (1% citric acid) exhibited the best results of final body weight, gain in weight, daily gain in weight (%) and specific growth rate. No mortalities occurred during the experiment period except a group that received the control diet, but a similar final weight, weight gain, and SGR were found on fish fed diet D2 (0.5% citric acid) and diet D3 (1.0% citric acid).

In this experiment, the specific growth rate did not agree with the range recorded by Kasi *et al*. (2012) who recorded a range of 1.42 and 6.59 for Cobia (*Rachycentron canadum*). In any experiment, the specific growth rate of any fish is subjected to various factors like weight gain, average feed intake, condition factor and water quality parameters of the culture media.

The means of condition factor were 1.73±0.05, 1.80±0.07 and 1.87±0.03 for Diet 1(control), Diet 2 (0.5 gm Citric acid) and Diet 3 (1.0 gm Citric acid) during the experimental period, respectively. No mortalities occurred during the experiment period except a group that received the control diet.

Effects on feed utilization parameters:

This study found that feeding diets containing different doses of dietary citric acid as a growth promoter supplementation (Diet 2 and Diet 3) in the diet of sex-reversed all-male Nile tilapia (*O. niloticus*) fingerlings during the experimental period, resulting in significantly higher feed utilization efficiency.

Based on the statistical analysis, the present results showed that both the control diet (Diet 1) and the diets containing different levels of dietary citric acid as a feed utilization supplementation (Diet 2 and Diet 3) in the diet of of sex-reversed all-male Nile tilapia (*O. niloticus*) fingerlings during the experimental period had no significant effect (*P>0.05*) on the feed intake (g diet/fish) (FI) and feed efficiency (FE) (g/ fish) , but had a significant effect (*P>0.05*) on the feed conversion ratio (FCR) (g/ fish) , protein efficiency ratio (PER) (g/fish) , protein retention (PR %) and protein productive value (PPV %).

Table 5: Effect of diets containing different doses of dietary citric acid on feed utilization parameters of Nile tilapia (*O. niloticus*) fingerlings.

Parameters	Experimental diets		
	Diet (1) Control	Diet (2)0.5 **gm** Citric acid	Diet (3)1 **gm** Citric acid
Feed intake (g diet/fish) (FI)	**74.66±0.23^{a}**	**75.35±0.16^{a}**	**75.19±0.73^{a}**
Feed conversion ratio (FCR) (g/ fish)	**1.25±0.01^{a}**	**1.22±0.01^{b}**	**1.21±0.02^{ab}**
Feed efficiency (FE) (g/ fish)	**0.80±0.01^{a}**	**0.82±0.02^{a}**	**0.83±0.01^{a}**
Protein efficiency ratio (PER) (g/fish)	**2.68±0.03^{b}**	**2.74±0.05^{a}**	**2.76±0.02^{a}**
Protein retention (PR %)	**8.66±0.19^{c}**	**10.14±0.03^{b}**	**11.04±0.06^{a}**
Protein productive value (PPV %)	**38.63±0.76^{c}**	**44.87±0.23^{b}**	**48.92±0.76^{a}**

a,b,c…. etc: means ± S.E. within the same row with different superscripts are significantly different (P≤ 0.05).

Supplementation of tilapia diets with the citric acid improved on feed intake (FI), feed conversion ratio (FCR), Feed efficiency (FE), and significantly ($P < 0.05$) increased protein efficiency ratio (PER), Protein retention (PR%) and Protein productive value (PPV %) (Table 5). The fish group fed D2 (0.5 gm Citric acid) and D3 (1 gm Citric acid) showed the higher (FI), (FE), (PER) , (PR %) and (PPV %) compared with the control treatment group.

Levels of organic acid. Furthermore, various concentrations of organic acids such as acetic acid, have been determined to have effects on the feeding behavior of *O. niloticus*.In addition, past research has also found that dietary supplementation of acetic acid at 10^{-5} M had no effect on fish feeding. (Lim *et al*. 2015) revealed that the benefits of the organic acid supplementation in the diet of fish may vary among fish and tend to be inconsistent, depend on the dietary ingredient, culture system, and water quality.

The largest increases in body weight and feed utilization were observed in groups that received diets with containing different doses of dietary citric acid supplementation (Diet 2 and Diet 3) in the diet of sex-reversed all-male Nile tilapia (*O. niloticus*) fingerlings; however, also significantly ($P > 0.05$) improved the growth performance of the fish. That supplementing diets with containing different doses of dietary citric acid supplementation stimulates growth has been reported previously in Nile tilapia (Hassaan, *et al.*,2013, 2014; Meshrf, 2014; Reda, *et al.*,2016) and rainbow trout, *Oncorhynchus mykiss* (De Wet, 2005).

The modes of action of organic acids appear to be different. Several hypotheses have been suggested to explain the effects of organic acids on enhancing nutrient utilization in terrestrial livestock, which include the following: lowering gastric pH leading to increased pepsin activation; lowering intestinal pH that might increase mineral solubilization resulting in increased mineral absorption; or as a result of decreased intestinal microbial activity that might otherwise utilize nutrients now spared for the host animal (De Wet, 2005).

Whole-body Proximate composition:

The proximate composition of the whole-body experimental fish at the end of the feeding trial as affected by diets containing different doses of dietary citric acid as a growth promoter supplementation are presented in Table 6.

It is evident from this Table that Body composition of dry matter, crude protein, crude lipid and ash contents were significantly affected by diets containing different doses of dietary citric acid as a growth promoter supplementation and it shows that dry matter, crude protein and ash increased (except crude lipid %) with increasing diets containing different doses of dietary citric acid as a growth promoter supplementation and decreased at control group diet (D1). It recommends that body

composition of treatment group diet 3 was adept to be better taking into consideration than other treatments.

Table 6: Effect of diets containing different doses of dietary citric acid as a growth promoter supplementation on the Whole-body proximate composition (dry matter (DM) basis %) of Nile tilapia (*O. niloticus*) fingerlings.

	Experimental diets			
Chemical analysis	Initial	Diet (1) Control	Diet (2)0.5 gm Citric acid	Diet (3)1 gm Citric acid
Dry matter (DM) %	**21.48±0.54**	**27.42±1.03^{c}**	**29.53±0.74^{b}**	**30.19±0.49^{a}**
Crude protein %	**54.44±0.78**	**52.98±1.0^{c}**	**55.31±1.08^{b}**	**59.43±0.50^{a}**
Crude lipid %	**20.87±0.93**	**28.85±0.66^{a}**	**26.84±0.13^{b}**	**25.02±0.45^{c}**
Ash %	**24.23±1.08**	**17.63±1.63^{b}**	**18.34±0.69^{a}**	**18.95±0.40^{c}**

Means in the same raw with different superscript letters are significantly different (P<0.05).

Incorporation of organic acids or their salts in O. niloticus diets significantly affected the proximate analysis of the treated fish. Proximate analysis of the present study showed that fish fed D3 (the basal diet supplemented by 1.0 % of citric acid) showed the highest protein and ash content and the lowest lipid content, while the control group (D1) showed the lowest protein and the highest fat content. Acidification of the experimental diets with each or organic acids seems to improve protein utilization and therefore improve protein gain. A negative relationship was also observed between protein and fat content of the whole fish body, as was previously found by Hassaan *et al*. (2014). Vielma *et al*. (1999) found that acidification of rainbow trout diets by citric acid increased whole-body ash content. The whole-body ash content of rohu (Labeo rohita) juveniles was not significantly affected by citric acid (Baruah, *et al*. 2007). Similar results were also reported by Van Weerd *et al*. (1999) in Clarias gariepinus and Vielma *et al*. (1998) in rainbow trout.

Hematology and Biochemical blood Parameters:

Haematological blood indices

Changes in the haematological parameters of Nile tilapia were affected significantly ($p < .05$) by citric acid levels as a growth promoter (Table 7).

As shown in Table 7 , hemoglobin (Hb), RBCs(10^6/mL) , WBCs (10^3/m l); PCV (%) ; MCV (Fl) ; MCH (Pg) ; MCHC (%);Platelets(10^3/m); Neu (%);Lemph (%) and Mon (%) were significantly ($P < 0.05$) lower in the control diet than the other experimental diets, which did not significantly differ from each other ($P > 0.05$). Values were significantly higher in fish from groups D2 and D3 in comparison with the control D1 treatment ($p < .05$), while the lowest values were noted in fish from the control D1.

Fish fed diet D3 (supplemented with 1.0 % citric acid) showed the higher serum total protein content, while the control group showed the lower serum haematological parameters compared with the other treatment ($P \leq 0.05$).

These could be attributed to the fact that the different levels of citric acid as a growth promoter used to increase the blood parameter values as a result of hemopiotic stimulation. These results supported the results of Marzouk *et al* (2008)**.**

Results in Table (7) showed that value of RBCs and WBCs counts were slightly significant ($P< 0.05$) in total count of RBCs in the fish fed diets containing supplementation citric acid. While WBCs counts significantly increased ($P< 0.05$) in all diets supplementation with citric acid than the control diet.

Table 7: Hematology and Biochemical blood Parameters of sex-reversed Nile tilapia (*O. niloticus*) fingerlings fed diets supplemented with different levels of citric acid.

Parameters	Experimental diets			sig
	Diet (1) Control	Diet (2)0.5 gm Citric acid	Diet (3)1 gm Citric acid	
Hematology:HB(g\dl)	4.84± 0.428^{c}	6.4 ± 0.383^{b}	6.8 ± 0.722^{a}	**
RBCs(10^6/**mL)**	**1.47±.015^{c}**	2.3±0.59 b	3.45±0.15a	**
WBCs (10^3/**m** l)	**57.10±1.10^{d}**	61 ± 2.37a	65.56 ± 3.2 a	**
PCV (%)	0.19500^{b}	0.25500^{a}	0.26000^{a}	**
MCV (Fl)	124.25 ± 2.59 c	133.35 ±2.05 b	165.45 ± 2.5 a	**
MCH (Pg)	36.51 ± 0.26 b	42.20±1.110a	42.95 ±1.25a	**
MCHC (%)	**25.74**± 0.7 a	24.33 ± 0.6 a	24.63 ± 0.92 a	***
Platelets(10^3/**m**)	**12.76** ± 0.53 c	13.9 ± 0.49 Ab	15.7 ± 0.12 a	***
Neu (%)	**28.5**±1.06b	29.61 ± 0.7 b	34.56±0.73A	***
Lemph (%)	**57.10±1.10^{c}**	66.55 ±0.54 b	68.66 ± 0.95 a	***
Mon (%)	**4.50±0.50^{a}**	4.66 ± 0.03 a	4.93 ±0.042 a	***
Biochemical :TP (g\dl)	**2.80±0.08^{c}**	**4.20±0.01^{b}**	**5.90**±2.250^{a}	***
ALT (U\l)	**25.50±**±10.860^{a}	**15.00±**10.772b	**13.50 ±** 0.860c	***
AST (U\l)	**44.00±**57.013^{a}	**35.00±**57.121^{b}	**32.00±**57.101^{c}	**

Values with different superscripts within rows are significantly different (*= P≤ 0.05 ,**= P≤ 0.01 ,***= P≤ 0.0001).

HB (g\dl) = Haemoglobin (HB g\dl),RBC (u\l) = total count of red blood cells (RBC ; 10^6 /ml) , WBC (u\l) = white blood cells count (WBC; 10^3/ml) , PCV (%)=Hematimetric variables (PVC) MCV (Fl)= Mean Corpuscular Volume (MCV), MCH (Pg)= Mean Corpuscular Hemoglobin (MCH), MCHC =Mean Corpuscular Hemoglobin Concentration (gdL-1), Platelets (10^3/ml) , Neu (%)=neutrophil,

Lemph (%)=lymphocyte, Mon (%)=monocyte , TP (mg\dl)= Total serum protein (TP) (g\dl) levels ALT (U\l)= Alanine aminotransferase ,AST (U\l)= Levels of serum aspartate amino transferase (AST)

Biochemical blood indices (immune response):

The immune response of fish as affected by treatments (Levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) activities, Data on some biochemical blood indices are illustrated in Table (7) and indicated that there were significant (p≤0.05) differences among tested dietary treatments in all criteria. And these results are illustrated in the results could be attributed to the immunomodulatory effect of citric acid as a growth promoter.

The results of the protein profile and Liver enzymes showed significant increases (P ≤ 0.05) in total Protein and Liver enzymes (ALT and AST). As presented in Table (7) the average value of total serum protein was significant increasing ((P< 0.05) with the increasing the addition citric acid levels and the lowest value showed in the control.

El- Dakar *et al.,* (2004) showed significant lower (P< 0.05) ALT and AST activities in the fish fed fennel seed meal in the diets. The results could be attributed to the immune-modulatory effect of biogen on the liver cells which activate the anabolic capacity of the hepatocytes to produce blood protein, particularly globulin (Ortuño *et al.*, 2002). It was also supported by the results of hepatic enzyme activity which decreased in *O.niloticus* kept on probiotic in comparison to the control group. These results were supported by the findings of (wang *et al*., 2008) and Mehrim (2009).

The estimation of serum transaminase activities (ALT and AST) is used as an indication of the amount of liver damage, as the elevated serum enzyme levels might be related to the degree of liver injury. Liver disease causes an increase in some serum enzymes by locking their elimination into the blood (Barraze *et al*. 1991).

CONCLUSION

In the present study, the beneficial impact of a moderate level (1%) of a dietary of citric acid surpassed that of in its ability to improve nutrient utilization and growth performance of Nile tilapia. This is particularly promising considering the current trend of increasing emphasis on food safety and traceability in aquaculture production leading to the reduction or ban in antibiotic use. these study demonstrate that water quality parameters, growth performance in terms of initial weight (g/fish) ,final body weight (FBW), weight gain (g/fish), average daily weight gain (ADWG) (%) , specific growth rate (SGR) (% day -1), condition factor (CF) and survival rate (SR) , feed utilization expressed as feed consumed (FC) (g/fish), food conversion ratio, FCR , protein efficiency ratio, PER , Net Protein Utilization (NPU%), the whole-body proximate composition in terms of dry matter, crude protein, fat (Ether extract-EE) and ash % and health status in Nile tilapia (*O. niloticus*) fingerlings during the experimental period (90 days) were affected significantly differences ($P<0.05$).

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