

## Effects of dietary malic acid on growth, nutrient utilization and serum biochemical indices of Nile tilapia (*Oreochromis niloticus*) fingerlings

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### ABSTRACT

A 90 days feeding trial was conducted to investigate the effect of dietary supplementation with malic acid on growth, nutrient utilization and some hematological parameters of Nile tilapia (*Oreochromis niloticus*). Three isonitrogenous (300 g CP kg<sup>-1</sup>) and isocaloric (14.90 MJ gross energy kg<sup>-1</sup>) diets were formulated. The control diet (D1) contained no added of malic acid. Two diets were supplemented with 0.5% (D2) and 1.0% (D3) malic acid. A set of 180 fish of *O. niloticus* L. mono-sex male fingerlings average initial weight of 7.56 ±0.06 g were randomly distributed into nine plastic tanks (0.45 m<sup>3</sup> for each) representing the three treatments studied. Twenty fish were randomly stocked into each tank with three replications for each treatment. At the end of the feeding trial, *O. niloticus* offered the control diet showed lower growth and feed utilization than all those fed malic acid supplemented diets. Fish fed the diet D3 showed the highest body weight (BW) weight gain (WG), specific growth rate (SGR), feed intake (FI), and the best feed conversion ratio (FCR) and protein efficiency ratio (PER). Supplementation of malic acid (5 g or 10 g kg<sup>-1</sup>) increased hemoglobin (Hb), hematocrit (Htc) and red blood cell count (RBCs) of Nile tilapia, but white blood cells (WBCs) of did not significantly (P>0.05) affected by malic acid supplementation. The highest level of Hb, Htc and RBC was recorded by fish fed 10 g kg<sup>-1</sup>. In the same direction supplementation the basal diet with 5 g or 10 g kg<sup>-1</sup> malic acid significantly increased the levels of serum Albumin u/L (P<0.001), Globulin (P<0.05) and total protein (P<0.001). The present observations suggest that, growth and feed utilization of *O. niloticus* can be enhanced with a supplementation of 1.0% malic acid in the diet.

**Key words:** growth, nutrient, organic acid, Nile tilapia.

### Introduction

Organic acids are an example of a group of additives which can play an important role in future in aquaculture diets. A number of studies, in cold-water species (Gislason *et al.*, 1996) and tropical species (Ramli *et al.*, 2005 and Petkam *et al.*, 2008), indicated a broad range of organic acids, their salts or mixtures can improve growth, feed utilization and disease resistance in fish. Organic acids in animal feed were initially used in piglets to complement their limited capacity to maintain a low gastric pH, which linked to problems with digestion (Easter, 1988). Antibiotic inhibit all microbial growth, whereas acidifiers are more selective in their activity (Cromwell, 1990). They can reduce harmful microorganisms and promote beneficial microflora colonization of the gastrointestinal tract (Mathew *et al.*, 1991).

As reviewed by Baruah *et al.*, (2008), the addition of organic acids in fish feeds may have many advantages; it may reduce the unwanted pathogenic microbial load in feed and the gut of fish; reduce toxic microbial metabolites by reducing the pathogenic microbes; enhance nutrient absorption due to proliferation of mucosal epithelium of the intestine; reduce the discharge of phosphorus in water thereby preventing aquatic pollution; reduce the risk of antibiotic residue in fish and prawns in those regions where antibiotics are used. Mineral absorption may

also be enhanced due to more solubilization of Ca, P and Mg etc from animal protein sources, especially in stomach-less fishes. Some organic acids especially citric, metacetic and acetic, are also added to pellets for storage effects and/or the improvement of feed utilization (Kumar *et al.*, 1997 and Sugiura *et al.*, 1998).

Malic acid is a four-carbon dicarboxylic acid that occurs naturally in many fruit organic acids (it was first isolated from apples), and it is synthesized commercially (Sniffen *et al.*, 2006). Malic acid has two stereoisomeric forms (L and D enantiomers), although only the L-isomer occurs naturally. This organic acid is used in foods as a flavor enhancer and for pH and microbial control, because it inhibits the growth of pathogenic bacteria and pathogenic fungi (Ricke, 2003). An exceptionally valuable nutritional property of this acid is that after oral administration, it stimulates the secretion of gastric juices and increases peristalsis. Small doses of malic acid have prebiotic properties, stimulating the development of non-pathogenic, acidophilic, and saprophytic bacteria, at higher doses, however, it can exhibit bacteriostatic and bactericidal properties (Salou *et al.*, 1991). Generally, the present study was carried out to investigate the effect of graded levels of malic acid on growth performance, feed utilization, and some physiological aspects of *Oreochromis niloticus*.

### Materials and Methods

### **Fish and culture technique**

The present study was designed to investigate the effect of malic acid (dicarboxylic acid) on growth, feed utilization, hematological and biochemical blood parameters of Nile tilapia, *O. niloticus*. Experimental fish were obtained from National Institute of Oceanography and Fisheries (NIOF), Elqanater Elkhairia, Qalubia, Egypt. Fish were acclimated to the experimental conditions in four tanks for two weeks at the laboratory of fish nutrition, Faculty of Agriculture, Benha University. During the acclimation period, fish were fed a control diet (300 g kg<sup>-1</sup> crude protein) at a rate of 3% of biomass, which provided of equal rations at 09:00 am and 3:00 pm for 2 weeks to adapt fish to the artificial diet and conditions of the trail. After the acclimatization, the experimental fish were randomly distributed into the experimental plastic tanks (0.45 m<sup>3</sup> for each) representing the three treatments studied. A set of 180 fish of *O. niloticus* L. mono-sex male fingerlings average initial weight of 7.56 ± 0.06 g were used in this trail. Twenty fingerlings were randomly stocked into each tank with three replications for each treatment. Underground water was supplied to each tank housed within an artificially illuminated room. Each tank supplied with automatic heater (200 watt) to maintain the water temperature (27-29°C). About one-third of water volume in each tank was daily replaced by aerated fresh water after removing the accumulated excreta. During the 90-days experimental period, tilapia were hand-fed with the respective diet 3% of biomass twice daily at 09:00 am and 3:00 pm. Every two weeks, total fish was taken from each tank then weighted and the amount of feed was adjusted according to the changes in body weight throughout the experimental period.

### **Water quality**

Water temperature was recorded daily at 1.00 pm using a mercury thermometer. Dissolved oxygen (DO) was measured at 07.00 am using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). A pH was estimated on morning by using a pH meter (Orion pH meter, Abilene, Texas, USA). Water temperature ranged from 27.20 to 29.25°C; dissolved oxygen (DO) ranged between 5.32 to 6.81 mg/l; pH values ranged between 8.04 to 8.30 and total ammonia ranged from 0.18 to 0.2 mg/l for the different treatments during the entire experimental period (90 days). All tested water quality criteria (temperature, pH value, DO and total ammonia) were suitable and within the acceptable limits for rearing Nile tilapia *O. niloticus* fingerlings (Boyd, 1990). These positive findings in water quality criteria related with good growth performance since there were no mortalities among all treatments. A photoperiod of 12-h light, 12-h dark (08:00–20:00 h)

was used fluorescent ceiling lights supplied the illumination.

### **Diet preparation**

Three isonitrogenous (293 g kg<sup>-1</sup> crude protein) and isocaloric (14.90 MJ kg<sup>-1</sup> gross energy) experimental diets were formulated and analyzed (Table 1). The experimental diets were supplemented with short chain organic acid (malic acid) with 0, 5 and 10 g kg<sup>-1</sup> diet, and represented D1, D2 and D3, respectively. All dry ingredients of soybean meal, corn gluten, yellow corn, wheat bran and rice polishing were blended for 5 mins and thoroughly mixed with fish oil. The ingredients were mixed well and made into dry pellets using a laboratory pellet mill (California Pellet Mill, San Francisco, CA, USA). The pellets (2-mm die) were dried for 4 h at 60°C and stored at -20 °C until use.

### **Growth performance and feed utilization parameters**

Records of live body weight (g) and body length (cm) were measured in all fish for each tank and registered every 14 days (two weeks) during the experimental period. Growth performance parameters were measured by using the following equations:

Weight gain (WG) = final weight (g) – initial weight (g)

Specific growth rate (SGR) =  $\frac{\ln W_2 - \ln W_1}{t} \times 100$

Where: Ln = the natural log, W<sub>1</sub> = first fish weight, W<sub>2</sub> = the following fish weight in grams and t = period in days.

Feed conversion ratio (FCR) = Feed ingested (g)/Weight gain (g)

Protein efficiency ratio (PER) = Weight gain (g)/Protein ingested (g)

### **Sample collection**

At the experiment termination, six fish were randomly selected from each treatment and anaesthetized with t-amyl alcohol to collect the blood sample. The remaining fish in the tank were maintained for subsequent analysis of gut microbial flora.

### **Chemical analysis of experimental diets**

Dry matter, crude protein, crude lipid and ash contents were determined by the standard AOAC (1995). Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Crude protein was determined by micro-Kjeldhal method, N×6.25(using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40-60°C). Ash was estimated by incineration at 550°C for 12 h. Crude fiber content was determined using the method of Van Soest *et al.* (1991). Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, crude fiber, ash then subtracting this sum from 100.

**Table 1.** Ingredient and proximate composition of the experimental diets (g kg<sup>-1</sup> dry matter)

	D 1	D 2	D 3
Soybean meal (44%)	450	450	450
Corn gluten	60	60	60
Yellow corn	170	170	170
Wheat bran	150	145	140
Rice polishing	100	100	100
Fish oil	40	40	40
Lysine	5	5	5
Methionine	5	5	5
Vit. & Mine. <sup>1</sup>	15	15	15
Vitamin C	5	5	5
Malic acid	-	5	10
<b>Chemical analysis %</b>			
Dry matter	89.53	89.01	89.00
Protein	29.32	29.26	29.18
Lipid	6.06	6.04	6.02
Ash	5.07	5.04	5.01
Fiber	6.47	6.37	6.32
NFE <sup>2</sup>	53.08	53.29	53.47
ME energy (kJ g <sup>-1</sup> feed) <sup>3</sup>	14.87	14.89	14.88

<sup>1</sup>Vitamin and mineral mix (mg or g / Kg diet): MnSO<sub>4</sub>, 40 mg; Mg O, 10 mg; K<sub>2</sub>SO<sub>4</sub>, 40 mg; ZnCO<sub>3</sub>, 60 mg; KI, 0.4 mg; CuSO<sub>4</sub>, 12 mg; Ferric citrate, 250 mg; Na<sub>2</sub> SeO<sub>3</sub>, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU;  $\alpha$ -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.

<sup>2</sup>NFE (Nitrogen free extract) = 100 - (crude protein + lipid + ash + fibre content).

<sup>3</sup>Metabolizable energy was calculated based on the standard physiological values of 18.8 kJ g<sup>-1</sup> protein, 13.8 kJ g<sup>-1</sup> carbohydrate, and 33.5 kJ g<sup>-1</sup> lipid (Brett and Groves 1979).

#### Determination of digestive enzymes activity

Samples of intestine from each treatment were immediately homogenized in 10 volumes (w v<sup>-1</sup>) of ice-cold physiological saline solution and centrifuged at 5000 g for 15 min at 4°C; then, the supernatant was stored for endogenous enzymes activity analysis (Furne *et al.*, 2008). Chymotrypsin and trypsin activities were determined according to Deguara *et al.* (2003), and lipase activities were estimated according to the method described by Zamani *et al.* (2009).

#### Blood sampling and hematological and biochemical indices

At the end of the experiment, blood samples were collected from the caudal vein of fish in all treatments and were divided into two portions. The first portion was collected with the anticoagulant 10% Ethylenediaminetetraacetate (EDTA) to measure hematocrit (Htc), haemoglobin (Hb), red blood cells (RBCs) and white blood cells (WBCs). Htc was determined as described by Reitman and Frankel (1957), Hb was determined using haemoglobin kits which is a standardized procedure of the cyanomethemoglobin method, and the total count of WBCs was carried out by the indirect method (Martins *et al.*, 2004). The second portion of the blood sample was allowed to clot at 4°C and centrifuged at 3,000 rpm for 10 min. The non-hemolyzed serum was collected and stored at -20°C until use. Levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were

measured according to the method described by Reitman and Frankel (1957), and serum creatinine was measured by calorimetric and enzymatic determine cation methods as described by Henry (1974). Total serum protein and albumin were determined according to Henry (1964) and Wotton and Freeman (1982), respectively. However, globulin was calculated by subtracting total albumin from protein according to Coles (1974).

#### Statistical analysis

All data were analyzed by using the software SAS, version 6.03 (Statistical Analysis System, SAS 1996). One-way analysis of variance (One-way ANOVA) was used to determine whether significant variation existed between the treatments. When overall differences were found, differences between means were tested by Duncan (1955) new multiple range test. Two-way ANOVA was used for analyzing the individual effects of malic acid and protease and the interaction between them. All differences were considered significant at P<0.05 and the results are presented as means with pooled standard error of the mean).

#### Results and Discussion

##### Growth performance and feed utilization

Results presented in Table 2 showed that the differences in fish initial body weight among different treatments were insignificant indicating that, the

experimental fish at the start of the experiment were randomly distributed. Supplementation of the basal diet with 5 or 10 g malic acid kg<sup>-1</sup> significantly (P<0.05) increased the final fish body weight (FBW) from 43.66 to 45.01 and 48.72 g/fish. In the same trend, weight gain (WG) values significantly (P<0.05) increased from 36.04 to 37.48 and 41.10 g/fish when the basal diet supplemented with 5 or 10 g malic acid kg<sup>-1</sup>, respectively. The same trend was also recorded for specific growth rate. Fish fed the diets supplemented with 5 or 10 g malic acid kg<sup>-1</sup> significantly (P<0.001) improved specific growth rate (SGR) from 2.08% (D1) to 2.13 (D2) and 2.23 day<sup>-1</sup> (D3). Nile tilapia fed the basal diet (D1) showed the lowest significant feed intake (62.66 g/fish), protein efficiency ratio (1.95) and the worst feed conversion ratio (1.74). Supplementation of the basal diet with 5 or 10 g malic acid kg<sup>-1</sup> significantly (P<0.05) increased feed intake from 62.66 to 65.40 and 65.30 g/fish, respectively. Supplementation of the basal diet with 50 mg protease did not alter the values of feed conversion ratio (FCR) and protein efficiency ratio (PER) while the highest level of malic acid (10 g kg<sup>-1</sup>) significantly improved FCR and PER.

Supplemented diets with 5 or 10 mg kg<sup>-1</sup> malic acid in the current study significantly improved growth performance and feed utilization compared with the basal diet (D1). Similarly, it was found in a previous study that potassium-diformate (0.2, 0.3, or 0.5%) significantly improved growth and FCR of *O. niloticus* (Ramli *et al.*, 2005). In another study, Hassaan *et al.*

(2014) showed that *O. niloticus* fed a supplement of 1% Ca-lactate had significantly higher growth, increased feed intake, and improved FCR and PER than the control group. Compared with the negative control, 1.5% of Ca-formate, Ca-propionate, Ca-lactate, and citric acid blend resulted in a numerical increase in WG by 11% (Petkam *et al.*, 2008). Rainbow trout (*Oncorhynchus mykiss*) fed 0.5 to 1.0 or 1.5% of formic acid+sorbic acid blend showed a significant improvement in BW, SGR, and FCR versus the control diet (De Wet 2005). Also, supplementation of sea bream (*Pagrus major*) diets with 1% of citric acid led to a significant improvement in fish growth (Hossain *et al.*, 2007). In another study, Silva *et al.* (2016) found that the highest final weights were in white-leg shrimp (*Litopenaeus vannamei*) fed diets supplemented with fumarate, succinate, butyrate, and propionate, increasing 53%, 46%, 38%, and 29%, respectively compared with the control diet. Also, shrimp that were fed diets with fumarate digested more feed and had a feed conversion ratio 23% higher than the control group. The highest improvement in FCR and PER occurred when diets were supplemented with mixture of organic salts, and the lower FCR and PER was observed when fish were fed the control diet. These positive effects of diet acidification could be the results of the reduction of pH in the stomach by mixture of organic salts that would increase pepsin activity and consequently improve protein digestion (Mroz *et al.*, 2000).

**Table 2.** Growth performance and feed utilization of Nile tilapia fed diets supplemented with

	D1	D2	D3	MSE	P values
	0 g kg <sup>-1</sup> MA	5 g kg <sup>-1</sup> MA	10 g kg <sup>-1</sup> MA		
IBW (g fish <sup>-1</sup> )	7.62	7.52	7.54	0.56	0.471
FBW (g fish <sup>-1</sup> )	43.66	45.01	48.72	1.12	0.031
WG (g fish <sup>-1</sup> )	36.04	37.48	41.10	1.01	0.041
SGR (% day <sup>-1</sup> )	2.08	2.13	2.22	0.26	0.001
FI (g fish <sup>-1</sup> )	62.66	65.40	65.30	1.12	0.013
FCR	1.74	1.74	1.58	0.32	0.004
PER	1.95	1.94	2.14	0.32	0.003

Data are presented as means ± standard mean error (SME) of triplicates.

Means followed by different letters in the same row are significantly different (P<0.05).

Hybrid tilapia (*O. niloticus* × *O. aureus*) fed diets supplemented with 10 g kg<sup>-1</sup> citric acid showed an increase in the activities of protease in the stomach by 29.6% but reduced the activities of protease in the intestine by 35.1% (Li *et al.*, 2009). The supplementation of diets with organic acids or their salts has been suggested to improve growth through improved digestion, absorption, and retention of a variety of nutrients and minerals. Organic acids also increase secretion of some enzymes such as proteases and modify digestive enzymatic activity and in vitro protein digestibility of white-leg shrimp, *L. Vannamei* (Silva *et al.*, 2016). Acidification of fish diets alters digestive enzymes activity and consequently nutrient utilization (Silva *et al.*, 2016). Soltan *et al.* (2017)

investigate the effect of incorporation of graded levels of two organic acids (malic acid, oxalic acid) blend and two organic acid salts (calcium lactate, sodium acetate) blend in Nile tilapia (*O. niloticus*) diets. Fish fed the diet supplemented by 1% malic+oxalic acids blend showed the highest BW (25.45 g) and control group showed the lowest BW (21.85 g) also supplementation of the basal diet by each of malic+oxalic acids blend or Na-acetate+Ca-lactate blend significantly improved the final BW of Nile tilapia compared with the control groups.

#### The activates of endogenous enzymes

In the present study addition of malic acid (5 g or 10 g kg<sup>-1</sup>) in Nile tilapia diets increased the activity of



endogenous enzymes chymotrypsin, trypsin and lipase (Table 3). In this context, **Li et al. (2009)** reported that dietary citric acid (10 g kg<sup>-1</sup>) increased the protease activity in tilapia stomach. Moreover, activities of amylase in hepatopancreas and gut were found to be elevated due to changes in the gut pH induced by dietary citric acid. This subsequently affected the release of cholecystokinin and exocrine secretions of the pancreas. Similarly, **Su et al. (2014)** reported a significant increase in intestinal protease activity of white shrimp fed 2 g kg<sup>-1</sup> citric acid compared with the control group. However, no significant differences were observed for the amylase activity among the shrimp fed graded levels of citric acid. **Castillo et al. (2014)** showed that supplementation of potassium diformate (KDF) at 15 g kg<sup>-1</sup> diet, especially citric acid, improved the growth performance in juvenile red drum, which they attributed in part to the enhanced activity of digestive enzymes. In this study, pepsinogen

activity was found to be greater in the stomach of fish fed the acidified diets. Nevertheless, the researchers suggested that pH of the diets was not the only factor affecting the synthesis of pepsin, as it might also be influenced by other factors. Dietary citric acid and KDF also resulted in higher activities of trypsin, lipase and amylase in fish. It was hypothesized that gut acidification by organic acids may increase secretin concentration which ultimately stimulate the secretion of pancreatic enzymes. **Silva et al. (2016)** reported that, white shrimp showed an increase in trypsin and chymotrypsin activity when fed diet supplemented with sodium acetate or propionate, but the activities of these enzymes were decreased in shrimp fed the diets supplemented with sodium lactate or citrate. Sodium formate, butyrate, fumarate and succinate did not alter the activities of these digestive enzymes in shrimp.

**Table 3.** Activities of endogenous enzymes of Nile tilapia fed diets supplemented with graded levels of malic acid.

	D1	D2	D3	MSE	P values
	0 g kg <sup>-1</sup>	5 g kg <sup>-1</sup>	10 g kg <sup>-1</sup>		
Chymotrypsin (U g <sup>-1</sup> tissue)	3.23	3.98	4.01	0.25	0.12
Trypsin (U g <sup>-1</sup> tissue)	0.69	0.76	8.10	0.31	0.004
Lipase (U g <sup>-1</sup> tissue)	718	811	850	9.36	0.005

Data are presented as means ± standard mean error (SME) of triplicates.

Means followed by different letters in the same row are significantly different (P<0.05).

#### Hematological parameters of *O. niloticus*

Results of Tables (4&5) showed that, supplementation of malic acid (5 g or 10 g kg<sup>-1</sup>) increased hemoglobin (Hb), hematocrit (Htc) and red blood cell count (RBCs) of Nile tilapia, but white blood cells (WBCs) did not significantly (P>0.05) affected. The highest level of Hb, Htc and RBC was recorded by fish fed 10 g kg<sup>-1</sup>.

Hematology is an important factor that could be considered for the fish diet quality assessment. **Svobodová et al. (1991)** reported that ichthohematology would be useful for the assessment of suitability of diets and feed mixtures, evaluation of fish conditions, determination of toxic effect of substances, as well as the diagnosis of disease. **Ologhobo, (1992)** reported that, one of the most common blood variables consistently influenced by diet is the hematocrit (Htc) and hemoglobin (Hb) levels. In the present study, addition of malic acid (5 g or 10 g kg<sup>-1</sup>) increased the levels of Hb, Htc and RBCs of Nile tilapia, but white blood cells WBCs did

not significantly (P>0.05) affected by malic acid supplementation. **Khajepour and Hosseini (2012)** showed that acidifying the diet of Juvenile Beluga (*Huso huso*) by adding 3% citric acid significantly increased (P<0.05) Hb and Htc values in Beluga. **Hassaan et al. (2014)** reported that diet supplemented with Ca-propionate or Ca-lactate at the different level (0.5, 1.0 or 1.5) significantly (P<0.05) increased hematocrit (Htc) and hemoglobin (Hb) values of Nile tilapia compared with fish fed the control diet and supplementation the experimental diets with 1.0% of each salt (Ca-propionate or Ca-lactate) released the highest Htc or Hb value compared with the other treatments. In another study, **Hassaan et al. (2017)** reported that Htc, Hb, red blood cell and white blood cell values of Nile tilapia fed diet supplemented with malic acid were significantly higher than control diet. Fortunately, the increase in hematological indices in the present study for fish fed the diet supplemented with malic acid increase the oxygen carrying capacity in the blood.

**Table 4.** Hematological parameters of Nile tilapia fed diets supplemented with graded levels of malic acid.

	D1	D2	D3	MSE	P values
	0 g kg <sup>-1</sup>	5 g kg <sup>-1</sup>	10 g kg <sup>-1</sup>		
Hb (g/dl)	13.25	14.63	15.98	0.98	0.001
Htc(%)	22.15	24.80	26.36	0.23	0.002
RBCs ×10 <sup>3</sup>	1.80	2.25	2.50	0.03	0.011
WBCs ×10 <sup>6</sup>	35.60	35.90	36.20	1.26	0.420

Data are presented as means ± standard mean error (SME) of triplicates.

Means followed by different letters in the same row are significantly different (P<0.05).

**Liver function and biochemical parameters**

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes are important liver enzymes indicator for liver health and function through controlling transferring amino group function of alpha-amino acids to alpha-keto acids. Large amount of ALT and AST are released into animal blood, mostly during liver cell damage (Kumar *et al.*, 2011). Increases in proteinogram levels are thought to be associated with a stronger innate response in fish (Jha *et al.*, 2007). Generally, the present study showed a significant reduction in the levels of ALT and AST due to acidification of the experimental diets with malic acid

and this may be due to improvement in general health of fish fed acidified diets (Table 5).

Our results showed a positive significant effect of acidification with malic acid of *O. niloticus* diets on serum total protein, albumin and globulin (Table 6). Globulin level is very often used as an indicator of immune responses and a source of antibody production. Baruah *et al.* (2007) also indicated a significant increase in total serum protein of Rohu (*L. Rohita*) when fed diet supplemented with citric acid. Nile tilapia fed diets containing malic acid exhibited a significant decrease in transaminases ALT and AST activity as well as improved values of total protein, albumin and globulin compared with fish fed the control diet (Hassaan *et al.*, 2017).

**Table 5.** Liver enzymes activities of Nile tilapia fed diets supplemented with graded levels of malic acid.

	D1 0 g kg <sup>-1</sup>	D2 5 g kg <sup>-1</sup>	D3 10 g kg <sup>-1</sup>	MSE	P values
ALT (u/L)	22.15	20.80	20.12	0.98	0.012
AST (u/L)	15.16	13.12	12.90	0.68	0.001
ALP (u/L)	139.20	132.33	130.10	2.68	0.002

Data are presented as means ± standard mean error (SME) of triplicates.

Means followed by different letters in the same row are significantly different (P<0.05).

**Table 6.** Total protein, albumin and globulin of Nile tilapia fed diets supplemented with graded levels of malic acid.

	D1 0 g kg <sup>-1</sup>	D2 5 g kg <sup>-1</sup>	D3 10 g kg <sup>-1</sup>	MSE	P values
Total protein u/L	3.79	4.60	5.21	0.36	0.001
Albumin u/L	1.78	2.12	2.56	0.25	0.032
Globulin u/L	2.01	2.58	2.56	0.12	0.023

Data are presented as means ± standard mean error (SME) of triplicates.

Means followed by different letters in the same row are significantly different (P<0.05).

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