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Effect of Dietary Organic Salts on Growth, Nutrient Digestibility, Mineral Absorption and Some Biochemical Indices of Nile Tilapia; *Oreochromis niloticus* L. Fingerlings

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Abstract: A total of 525 tilapia fingerlings with an average body weight (1.18±0.02 g) were divided into seven experimental treatments (Three replicates each). Seven isonitrogenous (30.66% crude protein) and isocaloric (19.26 MJ/kg gross energy) diets were formulated to contain 0% organic salts (control), diets 2-4 contained calcium propionate at 0.5%, 1.0% and 1.5% respectively, whereas diets 5-7 contained calcium lactate at 0.5%, 1.0% and 1.5% respectively. After 90 days of feeding, the fish fed diet containing 1.0% Ca-lactate recorded the higher ($P<0.05$) final body weight, weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), protein productive value (PPV),energy retention (ER) and the best feed conversion ratio (FCR) and higher apparent digestibility coefficients of crude protein (CP), ether extract (EE), gross energy (GE), Calcium (Ca), phosphorous (P), potassium (K) and sodium (Na) compared to fish fed the other diets. Blood serum showed an improvement in hemoglobin (Hb) content, hematocrit (Ht) values and total protein, while there was a decrease in aspartate aminotransferase (AST) and alanineaminotransferase (ALT) in fish fed 1% Ca-lactate.

Key words: Organic Salts · *Oreochromis niloticus* · Growth · Digestibility

INTRODUCTION

Both feed industry and food production sectors still suffer from losses due to the relation of feed with pathogenic bacteria and their related impacts in the animal, such as lower weight gains or increased mortality. The ban on the use of in-feed antibiotics in livestock in the European Union (EU) since 2006 puts more pressure on animal producers and poses a challenge to innovate animal nutrition. Thus, a greater interest has arisen in seeking alternatives to antibiotic substances that could inhibit pathogens and act as growth promoters [1]. In these context, organic acids or their salts have become a promising alternative feed additive for aquatic animal [2-4].

Consequently, feed safety is guaranteed by adding an organic acid or organic acid blended [5]. In the intestinal tract of aquatic animals, organic acids inhibit the growth of bacteria, especially gram-negative bacteria, by penetrating through the cell wall and releasing protons in the cytoplasm. Accordingly, the bacteria consume a large amount of adenosine triphosphate (ATP) to excrete protons in trying to keep the balance of intracellular pH, resulting in depletion of cellular energy and, subsequently, leading to death [6]. In addition, organic acids and their salts can improve the digestibility of the diet. In rainbow trout *Oncorhynchus mykiss*, dietary supplementation with 1% sodium diformate increased the digestibility of some nutrients, such as proteins and lipids [7].

In the first studies with organic salts (Lactate and propionate) in the aquaculture,it observed that the lactate supplementation of 1% in the arctic charr feed *Salvelinus alpinus*, increased growth andfeed efficiency [8]. Furthermore, study showed that using 0.5–3% citric acid favors nitrogen (N) and phosphorus (P) retention in sea bream (*Pagrus major*) [9], it also decreased the incidence of diarrhea during cultivation. Liebert *et al*. [10] showed that when 0.3% of sodium diformate was added to the diet of the tilapia fingerlings (*Oreochromis niloticus*), the protein

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efficiency ratio and protein retention significantly improved.

Moreover, studies showed that using 0.5-3% citric acid increase phosphorus (P) retention in sea bream (*Pagrus major*) [11], increases calcium (Ca) and P bioavailability in sturgeon (*Huso huso*) [12] and increases P retention in yellowtail (*Seriola quinqueradiata*) [13].

Some researchers have shown positive effects with dietary organic acids in improving growth rate and feed efficiency [5,14], but others have found nothing or negative responses [15-17]. The inconsistent results and highly variable responses may be due to several factors such as stage of growth, complexity of diet, source and level of organic acids and fish health status.

Available results related with the use of organic acids or their salts to improve the growth, feed efficiency, digestibility and mineral utilization in aquaculture are limited and not clear.Therefore the present study was carried out to investigate the effect of dietary calcium propionate and calcium lactate levels as organic salts on growth, nutrient digestibility, minerals absorption and biochemical indices of Nile tilapia, *Oreochromis niloticus* L. fingerlings.

MATERIALS AND METHODES

Fish Husbandry and Experimental Design: A set of 525 Nile tilapia, *Oreochromis niloticus* (L.) with an average initial weight (1.18±0.02g) were collected from the stock at Fish Research Station, Al Qanatir Al Khayryha, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt. Twenty-five fish was randomly stocked into each aquarium (180 L) with three replications per treatment. After stocking, to minimize stress of handling, all fish of each aquarium were weighed every 2 weeks and at the end of the feeding trial. The glass aquaria were supplied with de-chlorinated tap water. Aeration was continuously provided using an air blower. About one-third of water volume in each aquarium was daily replaced by new aerated fresh water after cleaning and removing of the accumulated excreta. A photoperiod of 12-h light, 12-h dark (08.00 to 20.00 h) was used. Fluorescent ceiling lights have supplied the illumination. During the 90-days experimental period, all fish were fed, initially, at a rate of 4 % of the total body weight daily and then gradually decreased to 3 % daily. Fish were fed twice a day (09:00 and 16:00 h) 6 days per week according to El-Saidy *et al*. [18]. Fish in each aquarium were sampled biweekly and the amount of feed adjusted accordingly.

Water temperature, dissolved oxygen, pH and total ammonia were monitored during the study, to maintain water quality at an optimal range required for Nile tilapia. Water temperature was recorded daily at 13.00 h using a mercury thermometer suspended at 30 cm depth. Dissolved oxygen (DO) was measured at 07.00 h using YSI model 56 oxygenmeter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and a pH at 09.00 h by using a pH meter (Orion pH meter, Abilene, Texas, USA). Total ammonia was measured three times a week according to APHA [19]. The water parameters in one of the following forms, averaged (±SD): The water temperature (28.1±0.3°C), dissolved oxygen (5.6±0.8 mg/L), pH (7.5±0.3) and ammonia (0.2±0.02 mg/L). All conditions of the trial evaluation in the present study were suitable and within the acceptable limits for rearing Nile tilapia fingerlings [20].

Experimental Diets: Seven isonitrogenous 30.66% crude protein and isocaloric 19.26 MJ/kg gross energy diets were formulated. The control diet contained no addedorganic acid salts. Diets 2-4 each contained calcium propionate at levels of 0.5%, 1.0% and 1.5%, respectively, while diets 5-7each contained calcium lactate at levels of 0.5%, 1.0% and 1.5%, respectively. Chromic oxide (Cr_2O_3) was used as an external marker at a level of 0.5% in each experimental formulated diet. The proximate chemical composition of the experimental diets is presented in Table 1. Calcium propionate CA $(C_2H_5COO)_2$ and Calcium lactate $(C_6H_{10}CaO_6)$ were purchased from an Adoia Pharmaceutical company (El-Sawah-Cairo-Egypt). The diets were processed by blending the dry ingredients into a homogenous mixture. In preparing the diets, dry ingredients were first ground to a small particle size (Approximately 250 μm). Ingredients were thoroughly mixed and then water was added to obtain a 30 % moisture level. Diets were passed through a mincer machine with 0.5-mm diameter of spaghetti like strands, which were dried under the sun rays for 8 h and stored in plastic bags in a refrigerator at (-20°C).

Growth Indices: Records of final body weight (FBW) (g) and final body length (FBL) (cm) of individual fish were measured in all fish for each aquarium at the initiation and the end of the feeding trial (90 days).Growth performance parameters were measured by using the following equations:

Condition factor (K)= $(W/L^3)\times 100$ Where: W = weight of fish in grams and L = total length of fish in "cm";WG = Final body weight (g) - Initial body weight

Table 1: Composition and proximate analysis of the experimental diets

	Experimental diets						
	Control	Calcium propionate (%)			Calcium Lactate (%)		
	0	0.5	1.0	1.5	0.5	1.0	1.5
Ingredients %	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Fish meal	16	16	16	16	16	16	16
Soybean meal	40	40	40	40	40	40	40
Yellow corn	28	28	28	28	28	28	28
Wheat bran	10.5	10	9.5	9	10	9.5	9
Soybean oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vit.& Min. mixtur[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Calcium propionate	0	0.5	1.0	1.5	0	0	0
Calcium lactate	0	0	0	0	0.5	1.0	1.5
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical analysis (determined on dry matter basis)							
Dry matter (DM)	89.41	89.01	89.11	89.00	89.21	89.10	89.19
Crude protein (CP)	30.85	30.75	30.45	30.64	30.60	30.68	30.71
Ether extract (EE)	5.68	5.65	5.63	5.71	5.66	5.68	5.69
Total carbohydrate[2]	57.51	57.37	57.38	56.71	57.15	56.67	56.25
Ash	5.96	6.23	6.54	6.94	6.59	6.97	7.35
GE (Mj/ kg diet)[3]	19.39	19.33	19.26	19.22	19.26	19.21	19.15
Mineral compositions							
Calcium (g/Kg)	8.68	10.93	14.93	18.92	14.15	16.14	18.14
Phosphorus (g/Kg)	9.76	9.70	9.64	9. 58	9.70	9.64	9. 58
Potassium (g/Kg)	10.47	10.41	10.35	10.29	10.41	10.35	10.29
Sodium (g/Kg)	1.69	1.69	1.69	1.68	1.69	1.69	1.68

[1]Vitamin and mineral mixture kg^{-1} of the mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, ascorbic acid, 500 mg, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulphate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I),

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

[3]Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj/g for protein, fat and carbohydrate, respectively according to[32].

(g); SGR% = (Ln FBW - Ln IBW)/t ×100; where: FBW is final body weight (g); IBW is initial body weight (g); Ln = natural logarithmic; t = time in days; Feed intake (FI) = total dry feed fed (g/fish);FCR = Feed intake (g)/weight gain (g); PER = weight gain (g)/protein intake (g). PPV% = (Protein gain (g)/protein intake (g)) ×100; FR% = (Fat gain (g)/fat intake (g)) ×100. ER% = (Energy gain (KJ) /energy intake (KJ)×100.

Digestibility Study: After 6 weeks of the feeding trial, expelled faecal materials (<6 h in water) were carefully siphoned and collected using a fine-mesh net. Only intact strands of faecal materials were collected as described previously [21]. Faecal samples collected from each tank were pooled, oven dried and finely ground before analysis of chemical composition and chromic oxide concentration. The chemical analyses were conducted according to Association of Official Analytical Chemists (AOAC)[22]. Chromic oxide was determined according to the procedure described by Furukawa and Tsukahara [23]. Apparent nutrient digestibility was calculated using equations of Schneider *et al.*[24] as following:

$$ADC_{\text{dietary nutrient}} = 1 - ((\text{Marker}_{\text{diet}}) / (\text{Marker}_{\text{faeces}}) \times (\text{Nutrient}_{\text{faeces}}) / (\text{Nutrient}_{\text{diet}}))$$

Hematological and Biochemical Parameters: At the end of the treatment period, ten fish were selected at random from each triplicate group, fasted overnight and anaesthetized using MS-222. MS-222 is tricainemethanesulfonate (TMS). MS-222 medium to anaesthetize fish was prepared by dissolving 40 mg of TMS powder in one liter of water. Whole blood was collected from the caudal vein of each fish using syringes rinsed in heparin (15 unit/ml), to determine the hematocrit and hemoglobin values. For separation of serum, blood

samples were withdrawn from the fish caudal vein, as before and transferred to Eppendorf tubes without anticoagulant. The blood samples were centrifuged at 3000×g for 15 min and the supernatant serum was collected and stored at -20°C until used for estimating the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by Reitman and Frankel [25], total protein (TP) and albumin (TA) by Wottonand Freeman [26], globulin by Coles [27] and creatinine by Henry [28].

Chemical Analysis of Fish and Experimental Diets: At the end of the experiment, a random group of five individual fish was sampled from each aquarium. They were pooled, ground, stored in polyethylene bags and frozen for subsequent body crude protein, lipid, moisture and ash content determination according to AOAC methods [22]. Total ash of the diets, fish and faecal matter was digested in a boiling nitric acid and perchloric acid mixture (2:1). After appropriate dilution Ca, K and Na contents were estimated by atomic absorption spectrophotometer while, P was estimated spectrophotometrically [22].

Statistical Analysis: Data were statistically analysed by ANOVA using SAS ANOVA procedure [29]. The data were submitted to one way classification variance analysis. Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed [30] at ($P<0.05$) level. All percentage data were arc-sin transformed prior to analysis [31]. however, data are presented untransformed to facilitate comparisons. Values are expressed as mean±standard error.

RESULTS

Growth Performance and Feed Utilization: Averages growth performance and feed utilization of Nile tilapia as affected by Ca-propionate and Ca-lactate are presented in Table 2. As described in this table fish fed the diet supplemented with 1.0% Ca-lactate released the highest final body weight (BW), weight gain (WG), specific growth rate (SGR), final body length (FBL) and the best condition factor. Although, supplementation of the basal diet by each of Ca-propionate or Ca-lactate with different doses (0.5, 1.0 or 1.5%) are significantly improved the final BW, BL, WG and SGR of Nile tilapia compared to control group, results also indicated that supplementation of the basal diet by Ca-lactate released the best the final BW, WG and SGR of Nile tilapia compared to Ca-propionate.

During the entire period (90 days) supplementation of tilapia diets by Ca-propionate and Ca-lactate were significantly ($P<0.05$) increased feed intake (FI) and decreased feed conversion ratio (FCR). Fish fed the diet supplemented with 1% Ca-lactate showed the highest of (FI), the best FCR, protein efficiency ratio (PER), protein productive value (PPV) and energy retention (ER) which significantly higher than the other diets.

Table 2: Growth performance and nutrient utilization of *O. niloticus* after 90 days of feeding Ca-propionate and Ca-lactate supplemented diets

	Experimental diets							
	Control	Calcium propionate (%)			Calcium lactate (%)			
	0	0.5	1.0	1.5	0.5	1.0	1.5	
	$Diet_1$	$Diet_2$	$Diet_3$	$Diet_4$	$Diet_5$	$Diet_6$	$Diet_7$	± SE
Initial Body weight (g)	1.18	1.19	1.20	1.17	1.20	1.16	1.17	0.02
Final Body weight (g)	16.90^{e}	17.77^{cd}	18.34^{b}	17.64^{d}	17.90^{c}	19.10^{a}	18.29^{b}	0.11
Final body length (cm)	9.32^{c}	9.77^{bc}	10.62^{a}	9.61^{c}	10.20^{ab}	10.60^{a}	10.40^{a}	0.17
Condition factor	2.41^{a}	2.06^{b}	1.57^{d}	2.02^{b}	1.71^{bc}	1.63^{d}	1.75^{bc}	0.08
Weight gain (g)	15.71^{e}	16.57^{cd}	17.14^{b}	16.46^{d}	16.70^{c}	17.74^{a}	17.12^{b}	0.07
Specific growth rate (%)	2.98^{c}	3.00^{bc}	3.03^{b}	2.98^{c}	3.01^{bc}	3.10^{a}	3.03^{b}	0.01
Feed intake (g/fish)	28.83^{c}	28.69^{c}	29.23^{ab}	29.51^{a}	27.90^{d}	29.51^{a}	28.96^{bc}	0.12
Feed conversion ratio	1.83^{a}	1.73^{c}	1.71^{cd}	1.79^{b}	1.67^{e}	1.64^{f}	1.69^{e}	0.01
Protein efficiency ratio	1.81^{f}	1.92^{d}	1.97^{c}	1.85^{e}	1.99^{b}	2.03^{a}	1.97^{bc}	0.01
Protein productive values (%)	27.23^{g}	29.93^{f}	32.79^{c}	31.43^{e}	31.95^{d}	35.04^{a}	34.02^{b}	0.02
Energy retention (%)	17.23^{f}	18.62^{e}	20.60^{c}	19.70^{d}	19.69^{d}	21.88^{a}	21.35^{b}	0.06

- Data are presented as means ± standard error (SE) of three replicates. Means in within same raw sharing the same superscript are not significantly different ($P>0.0$).

Table 3: Apparent dietary digestibility coefficients (%) of nutrients and minerals of Nile tilapia;*O. niloticus* after 90 days of feeding Ca-propionate and Ca-lactate supplemented diets

	Experimental diets							
	Control	Calcium propionate (%)			Calcium lactate(%)			
	0	0.5	1.0	1.5	0.5	1.0	1.5	
	$Diet_1$	$Diet_2$	$Diet_3$	$Diet_4$	$Diet_5$	$Diet_6$	$Diet_7$	± SE
Dry matter	86.16	86.10	87.50	86.55	86.16	87.60	86.63	1.47
Crude protein	87.77^{b}	88.06^{a}	85.97^{c}	86.07^{c}	86.95^{c}	88.58^{a}	88.56^{a}	0.07
lipid	86.88^{d}	89.16^{b}	89.96^{b}	89.60^{b}	87.72^{cd}	91.94^{a}	89.35^{b}	0.39
Total carbohydrate	48.32^{a}	44.01^{b}	47.60^{a}	44.29^{b}	48.03^{a}	38.73^{c}	43.54^{b}	0.40
Digestible energy	75.90^{c}	75.65^{cd}	77.01^{b}	76.44^{b}	74.62^{d}	77.80^{a}	75.60^{c}	0.32
Calcium %	27.90^{e}	28.51^{e}	29.86^{cd}	30.12^{bc}	29.34^{d}	30.78^{a}	30.94^{a}	0.23
Phosphorus %	31.14^{d}	34.36^{c}	35.55^{b}	35.87^{ab}	34.80^{c}	36.31^{a}	36.40^{a}	0.21
Potassium %	33.35^{d}	35.35^{c}	35.40^{c}	35.55^{c}	35.70^{c}	39.85^{a}	38.30^{b}	0.40
Sodium %	39.70^{d}	49.40^{c}	50.15^{bc}	50.85^{bc}	49.70^{c}	51.95^{a}	51.25^{b}	0.42

- Data are presented as means ± standard error (SE) of three replicates. Means in within same raw sharing the same superscript are not significantly different (P>0.05)

Table 4: Proximate analysis of Nile tilapia *O. niloticus* after 90 days of feeding Ca-propionate and Ca-lactate supplemented diets

	Experimental diets							
	Control	Calcium propionate (%)			Calcium lactate (%)			
	0	0.5	1.0	1.5	0.5	1.0	1.5	
	$Diet_1$	$Diet_2$	$Diet_3$	$Diet_4$	$Diet_5$	$Diet_6$	$Diet_7$	± SE
Dry matter (%)	24.80^{b}	25.73^{ab}	25.76^{ab}	25.97^{a}	25.43^{ab}	25.93^{a}	26.13^{a}	0.28
Crude protein (%)	54.11^{c}	55.72^{b}	56.72^{ab}	57.19^{a}	56.84^{ab}	57.77^{a}	57.25^{a}	0.43
Fat (%)	17.33	17.90	18.50	18.30	17.63	17.60	17.47	0.50
Ash (%)	17.10^{d}	17.90^{b}	18.27^{ab}	19.90^{a}	17.06^{d}	19.10^{a}	19.50^{a}	0.40
GE (Kcal/100g)	466.6^{c}	466.3^{c}	467.7^{bc}	466.7^{c}	469.2^{b}	480.1^{a}	479.8^{a}	0.66

- Data are presented as means ± standard error (SE) of three replicates. Means in within same raw sharing the same superscript are not significantly different (P>0.05).

Apparent Nutrients Digestibility: Apparent digestibility coefficients (ADC) for the different nutrient are presented in Table 3. There were no significant differences in the dry matter between experimental diets. The highest ADC for crude protein were recorded by fish fed diet containing 0.5% Ca-propionate, 1.0% and 1.5% Ca-lactate without significant (P>0.05) difference between these treatments, while, 1.0% Ca-lactate showed the highest ADC of lipid and digestible energy.

Apparent Absorption of Minerals (AAM): Results of Table 3 outlined the effects of Ca-propionate and Ca-lactate on apparent absorption of some minerals. Supplementation of the experimental diets by either of Ca-propionate or Ca-lactate significantly (P<0.05) increased apparent absorption (AAM) of Ca, P, K and Na compared with fish fed control diet. Fish fed the diet containing 1.0% Ca-lactate was recorded the highest AAM for Ca, P, K and Na.

Proximate Analysis of Whole Fish: Results of proximate analysis of the whole fish for the different treatments were outlined in Table 4. Dry matter (DM), Crude protein (CP), Ash and gross energy showed that fish fed the diets supplemented with 1.5% Ca-propionate, 1.0% Ca-lactate and 1.5% Ca-lactatehad higher DM content compared with the other experimental diets, while,1.0% Ca-lactate showed the highest crude protein and gross energy.

Hematological and Biochemical Parameters: Diet supplementation with Ca-propionate or Ca-lactate at the different doses (0.5, 1.0 or 1.5) were significantly increased hematocrit (Ht) and Hemoglobin (Hb) values of tilapia compared to fish fed the control diet (Table 5). The obtained results also indicated that the medium dose (1.0%) of each salt (Ca-propionate or Ca-lactate) released the highest Ht or Hb value of tilapia blood compared with the other treatments. ALT (U/L) and AST (U/L) followed the opposite trend. No clear trends were observed for total serum protein, albumin and globulin. No significant (P>0.05) difference was found in serum creatinine among all diet treatments.

Table 5: Hematological and biochemical parameters for Nile tilapia *O. niloticus* after 90 days of feeding Ca-propionate and Ca-lactate supplemented diets

	Experimental diets							
	Control	Calcium propionate (%)			Calcium lactate (%)			
	0	0.5	1.0	1.5	0.5	1.0	1.5	
	$Diet_1$	$Diet_2$	$Diet_3$	$Diet_4$	$Diet_5$	$Diet_6$	$Diet_7$	± SE
Hematocrit (%)	17.75^{f}	18.15^{e}	20.75^{a}	19.20^{c}	20.00^{d}	20.85^{a}	20.10^{b}	0.29
Haemoglobin (g/L)	7.60^{d}	8.00^{c}	10.55^{a}	9.20^{b}	8.55^{c}	10.60^{a}	9.10^{b}	0.35
ALT (U/L)	20.05^{a}	20.00^{a}	17.60^{c}	18.50^{b}	19.25^{b}	17.65^{c}	19.45^{a}	0.23
AST (U/L)	98.65^{c}	99.10^{b}	97.20^{d}	98.70^{c}	100.15^{a}	97.15^{d}	98.10^{c}	0.21
Total protein (g/dL)	2.78^{d}	2.75^{d}	2.85^{c}	$2. 92^{b}$	2.61^{e}	3.96^{a}	3.08^{b}	0.02
Albumin (g/dL)	1.42^{c}	1.43^{c}	1.455^{c}	1.43^{c}	1.333^{d}	1.70^{b}	1.74^{a}	0.01
Globulin (g/dL)	1.37^{c}	1.33^{d}	1.50^{b}	1.49^{a}	1.29^{e}	1.40^{b}	1.34^{c}	0.01
Createnine (g100/mL)	0.22^{a}	0.24^{a}	0.20^{a}	0.20^{a}	0.28^{a}	0.29^{a}	0.23^{a}	0.08

- Data are presented as means ± standard error (SE) of three replicates. Means in within same raw sharing the same superscript are not significantly different (P>0.05).

DISCUSSION

In the present study, supplementation of organic salt 1.0% Ca-lactate to the diet of *O. niloticus* improved body weight gain compared with fish fed the control diet without any added organic salts. Similar results have been obtained from several fish species fed organic acid or salt-supplemented diets. Increased WG and SGR of tilapa fed diet supplemented with organic salt; potassium di-formate [33], Arctic charrthan *Salvelinus alpines* fed 1.5% Na-lactate [15], carp juveniles *Labeo rohita* fed citric acid [21], Atlantic salmonused potassium di-formate [2], sea bream (*Pagrus major*) used 1% each of citric acid, malic acid and lactic acid [11] and marine shrimp *Litopenaeus vannamei* fed organic salt [4]. In contrast Petkam *et al.* [34] reported no significant improvement in the growth performance of Nile tilapia fed diet supplemented with organic acid blend or K-diformate. Accordingly, the effect of organic acids or organic salts seems to be dependent on fish species, sources and levels of organic acids, which probably reflecting differences in nutrient utilization or digestive processes.

Feed utilization parameters were paralleled with growth performance parameters for this study. The best FCR values were observed in 1% Ca-lactate and this value were significantly different from FCR values of control diet and other test diets.In practical terms, this implies that supplementation of fish diets with organic salt optimized protein use for the growth which can decrease the amount of feed necessary for fish growth, which could result in reducing production costs. From a nutritional point of view, the mechanism by which Ca-lactate, improved Nile tilapia growth in the current study may be attributed to the role in enhancing the population of beneficial microorganism in the digestive tract and inhibiting the potential pathogens, which improving intestinal microbial balance and enzyme activity and consequently improving feed digestibility and nutrient absorption. Improved FCR was reported in various fish species such as Arctic charr (*Salvelinus alpinus*) fed diet supplemented with1% Na-lactate [16], Indian carp (*Labeo rohita)* fed citric acid [21], *Oncorhynchus mykiss* fed blend consisting of formate and sorbate [14], tilapia fed potassium di-formate [33]. Moreover Lückstädt [2] reported that organic acids supplemented in fish diet reduced the pH level in the stomach and providing a remedythe problemsfeed digestion. Factors such as species and physiological age of the experimental fish [15], type and level of organic acids [8, 14], diet composition [17] and culture conditions [33] may be all influence the manifestation of the potential growth-promoting effects of dietary organic acids in aquaculture.

The apparent dietary digestibility coefficients of nutrients and minerals were enhanced by dietary inclusion of Ca-propionate and Ca-lactate in the present study with Nile tilapia *O. niloticus.*In this concern, Morken *et al*. [7] reported that Rainbow trout (*O. mykiss*), the addition of 1% sodium formate increased the digestibilityof lipids, ash and proteins. Baruah *et al*. [21] reporteda significant increase in phosphorus digestibility due to dietary addition of citric acid (30 g kg^{-1}) in the diets of *Labeo rohita* (Hamilton), Sugiura *et al*. [35] also observed an increase in the apparent availability of Ca, P, Mg, Mn and Fe in rainbow trout fed fish meal-based diets supplemented with citric acid. Recently, Khajepour and Hosseini [12] observed that an increase in protein digestibility and calcium (CA) and P bioavailability for sturgeon, *Huso huso* and increases P retention in yellowtail, *Seriola quinqueradiata* [13].

Increased minerals in this experiment attributed to Li *et al*. [36] who reported that the trace elements released by citric acid may have contributed to the increase on protease activity.Also, the same authorfound an increase activity of amylase in hepatopancreas and intestine, also, lowering diet and intestinal pH, which may increase mineral solubilization, as chelating agent binding up various cations along the intestine, which results in increased mineral absorption, or as a result of decreased intestinal microbial activity, which may otherwise utilize nutrients now spared for the host animal [14].

The highest value of Ash, fat and gross energy were observed at 1 and 1.5% calcium lactate as compared with control diet and othertest diets. In this respect, [37] found that acidification of rainbow trout diets by citric acid significantly ($P<0.05$) increased whole-body ash content. In a recent study, [13] indicated that, the supplementation of juvenile yellowtail, *Seriola quinqueradiata* diets by citric acid led to a slight increase in the whole-body ash content.

Hematology is an important factor that could be considered during the fish diet quality assessment. Svobodová *et al*. [38] 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. Similar to our results for some hematological and biochemical parameters, when Ca-propionate and Ca-lactate supplemented with based diet, Khajepour *et al*. [39] revealed that acidifying the diet of Juvenile Beluga (*Huso huso*) by adding 3% citric acid significantly increased ($P<0.05$) Hb content and Hct values in Beluga (*H. huso*) which may attribute to the effect of citric acid in the maximum liberation of Ca, P, Fe and Cu from the phytic acid complex. The same results obtained by Khajepour and Hosseini [12] for Beluga (*H. huso*) and Baruah *et al*. [21]for Rohu (*L. Rohita*).

Detection of high levels of ALP and ALT in blood gives information on the damage of organs and in particular of liver cells.The lower values of ALT (U/L) and AST (U/L) was recorded in the present study for Nile tilapia fed 1% Ca-propionate and 1% Ca-lactate compared to control diet indicating that healthy liver for Nile tilapia received organic salts.

ALT and AST enzymes are an 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 [40].

Fish fed diet supplemented with 1.0% Ca-lactate had significantly higher levels of TP compared to fish fed. According to the Wiegertjes *et al.* [41] increased in serum protein level is an indicator of innate immunity, which is considered as an important defense weapon of invertebrates and a fundamental defense mechanism of fish [42]. In the study carried out by Baruah*et al*. [21] on Rohu (*L. Rohita*) significant increase in total serum protein was found when fed diet supplemented with citric acid**.** There is no information demonstrate the effect of organic acid in kidney function of fish.The results of serum creatinine as an indicator of kidney function emphasized that acidified diets had no side impact on kidney in the present study.

CONCLUSION

The results of the present study demonstrated that adding organic acids to tilapia diets will markedly enhance growth, nutrient digestibility and hematological indices. It is also anticipated that under stressful, crowded and nonhygienic culture conditions, a greater positive growth response of tilapia can be observed when fed organic acid-supplemented diets.Longer termed feeding trials under less controlled culture conditions are currently being planned to further elucidate the mechanism of the potential growth-promoting effects of dietary organic acids in aquaculture.

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