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Effects of dietary pH and acid source on growth and feed efficiency of the Nile Tilapia, *Oreochromis niloticus* fry

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Abstract

Two feeding trials were conducted to investigate the effects of dietary pH (pH 2.5, 3.7, 4.6, 5.7-control, pH 7.0, and pH 8.0) and dietary acid source (acetic acid, citric acid, hydrochloric acid and control-no acid) on growth rate and feed utilization efficiency of the Nile tilapia fry. In addition, attractability of the diets at different pH and dietary acid sources were determined. Results of the first feeding trial showed that the feed with pH adjusted to 4.6 resulted in significantly highest final average body weight (FABW), weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER) and most efficient food conversion ratio (FCR) in the Nile tilapia fry. Survival was 100% in diets with pH 3.7 up to 7.0; the diet with pH 2.5 exhibited significantly lowest survival followed by the pH 8.0 diet. In the second feeding trial, diets that contained hydrochloric, citric or acetic acids that were used to adjust the pH to the optimal 4.6 resulted in higher FABW, WG, SGR, and PER values but lower FCR values than did the control diet; these parameters were not significantly different among the dietary groups. This study demonstrated that the dietary pH promoted growth and efficiency in the Nile tilapia fry and that the three acids that were used were similar in their effects on growth and efficiency. Furthermore, it was demonstrated that either acidifying the diet to pH 4.6 by an inorganic acid such as HCl or the provision of a dietary acidifier such as citric acid or acetic acid could enhance growth, feed efficiency and survival of the Nile tilapia.

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Introduction

High inclusion of plant protein in the diet of aquaculture fish serves as an alternative to fish meal in the fish feed industry to achieve aquaculture sustainability and maximized profitability in aquaculture production. Many studies have aimed to optimize the nutritional value of plant protein ingredients by using organic acids or their salts in combination with other additives such as exogenous digestive enzymes to eliminate the antinutritional factors (ANFs) and enhance the nutrients availability by modifying the stomach and intestinal pH to optimize the activities of the digestive enzymes (Hassaan et al., 2013; Dalsgaard et al., 2012; Hassaan et al., 2019a, Hassaan et al., 2019b). The addition of organic acids or their salts in tilapia diets has been reported to improve feed efficacy (Zhou et al., 2009; Ng et al., 2009; Hassaan et al., 2014; Abu Elala and Ragaa, 2015; Hassaan et al., 2018b). Organic acids or their salts are hypothesized to improve utilization of nutrient in fish based on the assumed mode of action, namely, i) they lower gastric pH leading to increase digestive enzymes and might increase mineral solubilization during digestion processes; or ii) they modify intestinal microbial activity that might create beneficial nutrient substance (De Wet, 2005; Lückstädt, 2008). To our knowledge, there is only a limited number of studies that have addressed the effect of pH on the growth of fish except for testing the efficacy of specific acids as feed additive such as malic acid in the Nile tilapia (Chen et al. (2017; Hassaan et al. 2018b), Crucian carp (*Carassius auratus*, Jing et al., 2013)).

Studies on supplementing an acidifier in the diet of tilapia demonstrated mixed results. Adding potassium diformate (KDF) did not promote growth and feed efficiency in hybrid tilapia *Oreochromis niloticus* x *O. aureus* compared with the group with no supplementation at all. Ng et al. (2009) fed red hybrid tilapia, *Oreochromis* spp., with various levels of a novel organic acid blend or with 2 g kg^{-1} of KDF. Their data showed that dietary organic acids can exert strong anti-microbial effects and have the potential to exert beneficial effects on growth, nutrient utilization and disease resistance in tilapia. In another study, Hassaan et al. (2014) fed Nile tilapia *Oreochromis niloticus* with a diet either with no acidifier as control diet, with various levels of calcium propionate or with various levels of calcium lactate. After 90 days of feeding, the fish fed diet containing 1.0% Ca-lactate recorded the higher protein productive value (PPV) and energy retention (ER) values and 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. Soltan et al. (2017) fed groups of Nile tilapia fingerlings with a control diet that did not contain any organic acids nor organic salts, or diets containing various levels of malic acid + oxalic acid blend (OAB) or with diets containing various levels of calcium lactate + sodium acetate blend (OSB). Their results reveal that all fish fed diets containing 1% of any combination of acid blends were superior to those fed with the control diet in terms of final body weight, weight gain, specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio. In a more recent study of Hassaan et al. (2020), the combination of malic acid and exogenous digestive protease enzymes at various levels of supplementation were evaluated in the Nile tilapia. The results suggest that adding exogenous sources of protease enzymes in combination with malic acid at 5 g kg^{-1} in plant protein-based diet improved growth performance, feed utilization efficiency, digestive enzymes and health status of Nile tilapia. They speculate that the decrease of stomach pH with the addition of organic acid caused an increase in pepsin activation and mineral absorption, which could be the reason of the improvement of growth and feed efficiency; their observation agrees with the observations of De Wet (2005) and Ng et al. (2009).They hypothesize that this improvement may be due to an induced optimization of intestinal pH by malic acid, which in turn helped to pave the exogenous protease activity. El Naby et al. (2019) incorporated sodium butyrate (SB) in a practical diet for Nile tilapia to evaluate its effects on the performance, immunity, and challenge of Nile tilapia against *A. hydrophilainfection*. Their data revealed that dietary SB showed stimulating-effects on fish growth, feed utilization, and innate immunity of Nile tilapia.

In the present study, our aim was to (1) determine the optimal dietary pH value, using the inorganic hydrochloric acid, that results in the best growth performance, feed utilization efficiency and survival of the Nile tilapia fry (experiment 1) and (2) determine further which among the three acids, one inorganic and two organic acids (hydrochloric, acetic, and citric acids) could enhance the growth parameters of the Nile tilapia fry (experiment 2) at the optimal pH determined in experiment 1.

Materials and Methods

The experiments were conducted at the laboratory of the National Institute of Molecular Biology and Biotechnology (NIMBB), University of the Philippines Visayas, Miagao, Iloilo, Philippines.

Experimental tilapia and set up

Two sets of one thousand (1000) sex reversed Nile tilapia (*Oreochromis niloticus*) fry were procured from the Southeast Asian Fisheries Development Center-Aquaculture Department (SEAFDEC-AQD) in Tigbauan, Iloilo, acclimatized to the basal diet and to the laboratory conditions for 10 days. Four hundred fifty and three hundred tilapia fry were randomly stocked in eighteen and twelve 60L tanks (25 fry $tank^{-1}$) for the experiments 1 and 2, respectively. The experimental diets were fed to three replicate groups of Nile tilapia fry three times a day. At the start of the experiments and every 15 days, fish were bulk-weighed, and satiation feeding was measured at every start day of the week and made as the basis for the feeding rate for the rest of the week; this was made as a routine cycle for the estimation of daily feeding rate until termination on the 56th day. The feeding trial was conducted in a closed recirculating system in which approximately 70% of the water in the system was replaced every two days. Uneaten feed and feces were siphoned-off each morning before the first feeding. Chlorinated tap water used for replacement (100 ppm NaClO) was dechlorinated by letting it stand while being aerated for 3 days. Water quality indices were monitored periodically: temperature and pH were measured twice a day, dissolved oxygen (DO) twice a week, and nitrite and total ammonia weekly employing commercial water quality kits.

Feed preparation

The basal diet (**Table 1**) was composed of Peruvian fish meal as the primary protein source in experiment 1 while Danish fishmeal in the second experiment; the rest of the ingredients were squid meal, shrimp meal, soybean meal, rice bran, cornstarch, soybean oil, vitamin premix, trace mineral premix and carboxymethylcellulose (CMC). All of these ingredients were purchased from SEAFDEC-AQD in Tigbauan Iloilo, Philippines and passed through 150µm sieve before use. The composition of the experimental diets is shown in Tables 1 and 2 in experiments 1 and 2, respectively.

Table 1 Composition of experimental diets containing different dietary pH levels fed to the tilapia fry (*Oreochromis niloticus*) for 56 days.

Feed Ingredients	Composition (gram)
Peruvian fishmeal	200.0
Squid meal	130.0
Shrimp meal	130.0
Soybean meal	280.0
Rice bran	120.7
Cornstarch	50.0
Soybean oil	30.0
Vitamin premix	21.7
Trace mineral premix	21.6
CMC	16.0
Total	1000.0
	Proximate Composition (%, dry weight basis)
Crude protein	49.3
Crude fat	8.7
Crude fiber	
Ash	10.8
Moisture	8.6

Table 2 Composition of experimental diets with pH adjusted to 4.6 by adding one of the 3 organic acids and fed to the Nile tilapia fry (*Oreochromis niloticus*) for 56 days.

	Treatments (% composition)			
Feed Ingredients	Control	Acetic acid	Citric acid	Hydrochloric acid
Danish fishmeal	180.0	180.0	180.0	180.0
Squid meal	130.0	130.0	130.0	130.0
Shrimp meal	130.0	130.0	130.0	130.0
Soybean meal	280.0	280.0	280.0	280.0
Rice bran	120.7	120.7	120.7	120.7
Cornstarch	50.0	50.0	50.0	50.0
Soybean oil	30.0	30.0	30.0	30.0
Vitamin premix	21.7	21.7	21.7	21.7
Trace mineral premix	21.6	21.6	21.6	21.6
CMC	16.0	16.0	16.0	16.0
Acids	0.0	20.0	20.0	20.0
Distilled water	20.0	0.0	0.0	0.0
Total	1000.0	1000.0	1000.0	1000.0
	Proximate Composition of the basal diet (% dry weight basis)			
Crude protein		47.7		
Crude fat		8.1		
Crude fiber				
Ash		11.6		
Moisture		10.3		

Experimental diet and pH adjustment

The formulated feeds in experiment 1 was adjusted to pH 2.5, 3.7, 4.6, 5.7 (control), pH 7.0 and pH 8.0. In experiment 2, the diets were prepared using three different acids , namely, acetic acid, citric acid, and hydrochloric acid to adjust the pH of the diets to the optimal pH level for growth and efficiency observed in experiment 1 which was pH 4.6. The sieved ingredients were manually mixed by shaking them thoroughly inside a big plastic bag. pH adjustment of every diet was done first using a small amount of sample (10 g) of the mixed powder mixed with distilled water and made up to 30 ml; pH was measured and adjusted to the desired pH by adding either an aqueous HCl solution or an aqueous NaOH solution in experiment 1, or one of the 3 acids in experiment 2. Following estimation on a small sample, adjustment of pH was done on a larger scale but final pH was further measured and adjusted. The resulting dough was pressed flat on an oven tray, cut into noodle-like forms while still a dough and was oven-dried for 12 h at 80°C. The dried product was crumbled to appropriate size, stored in plastic containers at 4°C until use. The pH of a sample of the dried diet was again measured in the manner described above for the testing and pH adjustment of a small sample; this was for final verification.

Attractability test

Ten runs were conducted using a custom-built rectangular glass tanks with multiple chambers as patterned after Suresh et al. (2011) shown in Figure 1. Six runs were performed in experiment 1 while 4 in experiment 2; each treatment was replicated three times on separate days. Each tank consisted of 3 major chambers (acclimatization chamber, middle chamber, and feeding chamber). The three chambers were separated with removable glass shutters. The feeding chamber consisted of 5 sub-chambers, each with a 6 × 5 cm opening so that the juvenile tilapia had access to the feeding chamber.

Each tank was filled with 40 L freshwater (5 parts thousand^{-1}, $^{0}/_{00}$) and was set up in a laboratory room in which it received only artificial light. Ten tilapia fry (ABW=0.2 g) were randomly stocked in the acclimatization chamber and allowed to acclimatize for 1 h. Two g of each test diet were placed in each of the 5 chambers randomly. The shutter was removed 2 min after feed placement to allow the fish to have access to the feed. Feed preference was quantified by counting the number of tilapia fry in the feeding chamber at 1, 3, and 10 min following shutter rising. Percent attractability of each dietary treatment was estimated as the number of tilapia juvenile that entered the feeding chamber of a specific experimental diet divided by the total number of tilapia placed in the acclimatization chamber multiplied by 100.

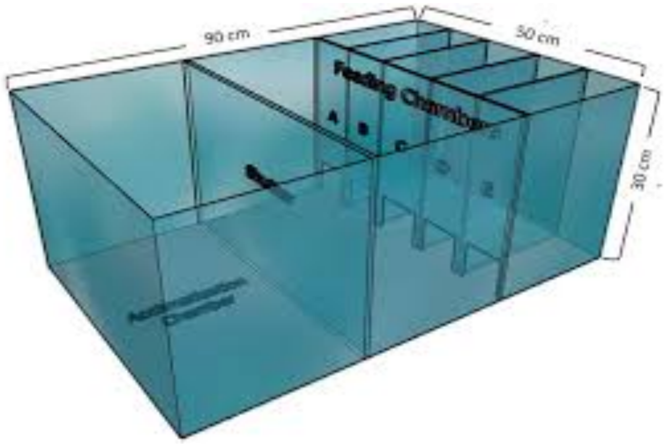

Figure 1 Schematic diagram of the tank used for the attractability test

Growth performance parameters.

Growth performance and feed efficiency were calculated using the following formula: Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR). These parameters were estimated as follows:

$$WG\ (g) = FABW - IABW$$

$$FCR = FI\ (g)/WG\ (g)$$

$$PER = (FABW - IABW)/(FI \times FP)$$

$$SGR\ (\%\ day^{-1}) = 100 * (Ln\ FABW - Ln\ IABW)/D$$

$$Survival\ (SR, \%) = 100 * \text{Final count of fish}/\text{Initial count of fish}$$

Where: FABW= Final average body weight (g) of individual fish; D= days of culture; IABW = initial average body weight (g) of individual fish; FP= Feed protein (in decimal); FI = total feed intake of individual fish for the whole duration of the experiment.

Statistical analysis

Statistical package for Social Sciences (SPSS) version 20 software was used to perform statistical analysis. Data (survival, weight gain, specific growth rate, and feed conversion ratio) were presented as mean ± standard error of the mean (SEM). Each set of data were tested for homogeneity of variance and normality of distribution, and upon passing, the data was subjected to one-way analysis of variance (ANOVA) at $\alpha = 0.05$. Post hoc analysis was done following ANOVA using Duncan's Multiple Range Test (DMRT) to identify differences between independent factors. The percentage numbers of tilapia fry from attractability test as well as the survival rates were subjected to arcsine transformation, then to the two tests of homogeneity of variance and normality of distribution before subjecting to one-way ANOVA.

Results

Water quality

The mean water quality parameters in the whole duration of the experiments were recorded as follows: water temperature range was 21-29°C; total ammonia nitrogen (TAN) range of 0.1-0.2 mg L^{-1}; average water salinity at 5 g L^{-1}, pH range of 7.5- 8.5 and nitrite range of 0.0-0.2 mg L^{-1}.

Attractability test

Results showed that the pH 4.6 diet attracted significantly most number of Nile tilapia juvenile than died diets with pH below or above this level (**Table 3**). Furthermore, diets containing hydrochloric acid, citric acid and acetic acid which were used to adjust the diet pH to 4.6 in Experiment 2 resulted in significantly higher attractability rates than did the control diet (**Table 4**). It was apparent that attractability was more related to the dietary pH than to the source of the acid.

Table 3 Percent of tilapia fry attracted to the basal and of test diets in Experiment 1 after 10 min of feed placement in the feeding chamber

Dietary pH	% shrimps attracted
2.5	8.3±0.8^{a}
3.7	13.3±0.8^{b}
4.6	28.3±0.8^{e}
5.7	18.3±0.8^{d}
7.0	16.7±0.8^{cd}
8.0	15.0±0.0^{bc}

All values are expressed as mean±SEM. Means in the same column sharing the same subscript are not significantly different (p>0.05).

Table 4 Percent of tilapia fry attracted to the basal and test diets containing various dietary acid sources in Experiment 2 after 3 min of feed placement in the feeding chamber

Dietary acid source	% shrimps attracted
Acetic acid	28.3±1.7^{b}
Citric acid	30.0±2.9^{b}
Hydrochloric acid	30.0±0.0^{b}
Control-No acid	11.7±1.7^{a}

All values are expressed as mean±SEM. Means in the same column sharing the same subscript are not significantly different (p>0.05).

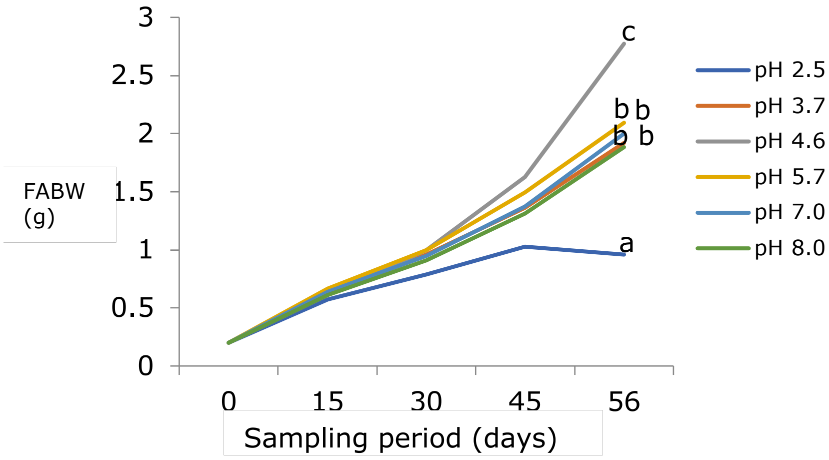

Figure 2 Periodic weight gain of tilapia fry fed the different pH level of feeds. FIBW= final individual body weight

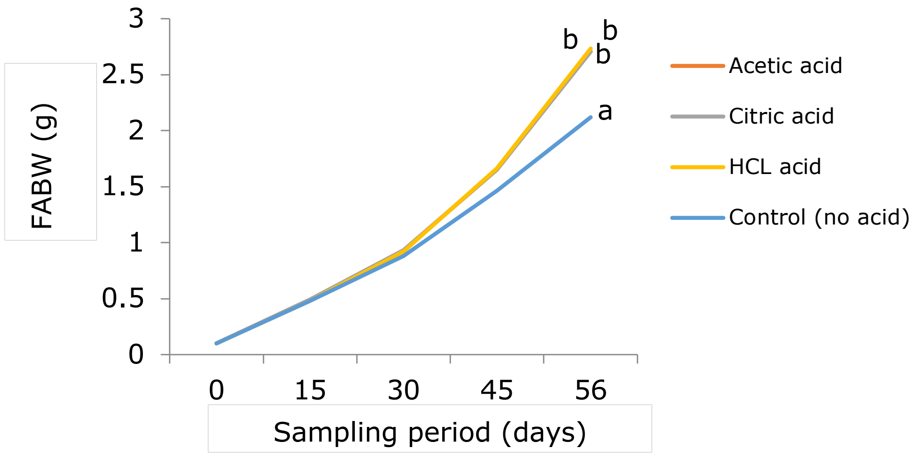

Figure 3 Periodic weight gain of tilapia fry fed to different acid of feeds. FIBW= final individual body weight; HCL=Hydrochloric

Growth, feed efficiency and survival

Results of the periodic sampling showed that starting at day 30 after stocking and until termination on day 56 the FABW of the Nile tilapia significantly highest in tilapia fed diets whose feed was adjusted to pH 4.6 and higher than those fed the other diets (**Figure 2**). The FABW of fish fed diets at pH levels below or above pH 4.6 except that at pH 2.5 were significantly below that of fish fed the pH diet at pH 4.6 and were not significantly different from each other. Tilapia fed the pH 2.5 diet exhibited significantly the lowest FABW. At the termination of the experiment, tilapia fed the pH 4.6 diet exhibited significantly the highest ABW, WG, and SGR values than those fed diets adjusted to other pH levels. However, diets at pH levels 3.7, 5.7, 7.0 and 8.0 were not significantly different from each other. The pH 2.5 diet resulted in the worst growth parameter values. FCR value was lowest in the pH 4.6 group and the PER value was significantly highest among all treatment groups demonstrating that the diet at pH 4.6 resulted in the conversion of nutrients into flesh (**Table 5**). Survival rates in fish fed the pH 2.5 and 8.0 diets exhibited a significantly lowest survival rates among the other treatment groups. In experiment 2, FABW of diets adjusted to pH 4.6 supplemented with any of the three acids were significantly higher than that of tilapia fed the control diet in which no acid was added and the pH not adjusted (**Figure 3**). At the termination of the experiment 2, all tilapia groups fed diets with any organic acid exhibited significantly higher FABW, WG, and SGR values than those fed the control diet. Feed efficiency of the diets with any one organic acid and pH 4.6 were with significantly lower FCR and higher PER values as compared to fish fed the control diet (**Table 6**). No mortality was observed in all treatment groups in experiment 2.

Table 5 Growth, nutrient utilization efficiency and survival of the Nile tilapia fry after 56 days of feeding diets adjusted to various dietary pH levels

pH level	IABW	FABW	FI	WG	SGR	FCR	PER	SR
2.5	0.2	1.0±0.1[a]	2.7±0.1	0.8±0.1[a]	1.5±0.1[a]	3.6±0.3[c]	0.3±0.1[a]	86.7±2.7[a]
3.7	0.2	1.9±0.0[b]	3.2±0.1	1.7±0.0[bc]	3.3±0.1[bc]	1.8±0.0[b]	0.8±0.0[b]	100.0±0.0[c]
4.6	0.2	2.7±0.2[c]	3.4±0.2	2.6±0.2[d]	4.9±0.3[d]	1.3±0.0[a]	1.3±0.0[c]	100.0±0.0[c]
5.7	0.2	2.1±0.0[b]	3.3±0.0	1.9±0.0[c]	3.7±0.1[c]	1.8±0.0[b]	0.9±0.0[b]	100.0±0.0[c]
7.0	0.2	2.0±0.1[b]	3.2±0.1	1.8±0.1[bc]	3.5±0.1[bc]	1.8±0.1[b]	0.9±0.0[b]	100.0±0.0[c]
8.0	0.2	1.9±0.1[b]	3.1±0.1	1.6±0.0[b]	3.3±0.2[b]	1.9±0.0[b]	0.8±0.1[b]	92.0±2.3[b]

Initial average body weight (IABW), average body weight (ABW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PER), survival rate (SR). Means in the same column sharing the same superscript are not significantly different (p>0.05).

Table 6 Growth, nutrient utilization efficiency and survival of tilapia fry after 56 days of feeding diets adjusted to pH 4.6 by adding various dietary organic acids.

Acids	IABW(g)	ABW(g)	FI(g)	WG(g)	SGR(% day^{-1})	FCR	PER	SR(%)
Acetic	0.1	2.7±0.1[b]	3.8±0.1	2.6±0.0[b]	4.7±0.1[b]	1.5±0.3[a]	1.5[b]	100.0±0.0
Citric	0.1	2.7±0.1[b]	3.8±0.1	2.6±0.0[b]	4.7±0.1[b]	1.5±0.0[a]	1.5[b]	100.0±0.0
Hydrochloric	0.1	2.7±0.1[b]	3.8±0.1	2.6±0.0[b]	4.8±0.2[b]	1.4±0.0[a]	1.5[b]	100.0±0.0
Control	0.1	2.1±0.1[a]	3.5±0.1	2.0±0.1[a]	3.6±0.2[a]	1.8±0.1[b]	1.2[a]	100.0±0.0

Initial average body weight (IABW), average body weight (ABW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PER), survival rate (SR). Means in the same column sharing the same superscript are not significantly different (p>0.05).

Discussion

The global shortage in fish meal supply is pushing feed researchers and manufacturers to use plant protein ingredients in their formulation of feeds. But the challenges that they meet are the presence of antinutritional factors and imbalanced amino acid profiles in plant protein sources. These two factors lead to poor digestibility and growth performance and result in a ceiling limit in their inclusion in aquafeeds. The present study aimed to investigate the effects of varying dietary pH by using inorganic acid HCl that could optimize the intestinal pH of Nile tilapia. The results of the present study showed that the best growth performance and feed utilization was observed in Nile tilapia fed the pH 4.6 diet; using any of the three acids tested, namely, acetic, citric or HCl to adjust the pH level to pH 4.6 resulted in similar growth and efficiency performance. The evaluation of growth rates and feed efficiency of the Nile tilapia in relation to dietary pH in the present study is an indirect comparison of the general physiological conditions in which the total digestive enzyme activities according to their corresponding substrates. The decrease of stomach pH with the addition of acid in the diet might have caused an increase in pepsin activation and mineral absorption that presumably led to the improvement of growth and feed utilization efficiency (De Wet, 2005; Ng et al., 2009).

The similar improvement on growth performance and feed utilization brought about by acetic, citric and hydrochloric acids demonstrated that it was the dietary pH and not any particular acid was the main factor in the improvement. There are two gastric acidification strategies that have been reported in vertebrates: (1) those that maintain a permanent acidic environment in the stomach which is not affected by the presence or absence of a meal (e.g. mammals and birds), and (2) those that maintain a neutral pH in the lumen of the stomach between meals and becomes slightly acidic after a meal (Papastamatiou and Lowe, 2005). Most teleostean fish that have been studied so far exhibited this second strategy (Hlophe et al., 2014; Nikolopoulou et al., 2011; Yúfera et al., 2004, Yúfera et al., 2012; Solovyev et al., 2016). The first strategy have been observed in cobia juveniles (Yufera et al., 2019), rainbow trout *Oncorhynchus mykiss* (Bucking and Wood, 2009), southern bluefin tuna *Thunnus maccoyii* (Leef et al., 2012) and in some elasmobranchian species (Papastamatiou and Lowe, 2005; Papastamatiou et al., 2007); these are strictly carnivorous and the observations were from studies which involved comparison of fed and fasted fish. Erratic daily feeding by changing randomly the moment of feed delivery every day may also alter the daily pattern from neutral/acid alternation to permanent acidification and this has been demonstrated in gilthead seabream *Sparus aurata* (Montoya et al., 2010). A constant acidic gastric pH enables this voracious species to be always ready to activate pepsinogen to start the hydrolysis of the ingested prey. In the Nile tilapia in the present study, feeding the fish continually with acidic diets at various pH levels could have forced a condition of permanent acidification of the lumen (i.e. the first strategy of gastric acidification, Yufera et al., 2019). This implied an environment in which the synthesis of digestive enzymes or activation of already existing digestive enzymes readily occurred for increased nutrient utilization.

In conclusion, the optimal dietary pH value of adjusted to pH 4.6 diet caused better for growth and feed efficiency in the Nile tilapia under laboratory conditions. Any acids at optimum pH 4.6 diet increased attractability, growth and feed efficiency to Nile tilapia fry.

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