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Original Article

Improving the growth, feed efficiency and hematological indicators of Nile tilapia fingerlings *Oreochromis niloticus* using dietary lactic acid supplementation with different feeding routines

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ABSTRACT: A 42-day trial 2 × 2 factorial study was conducted to investigate the importance of using lactic acid (LA) as a feed additive in different feeding regimes on the growth, feed and blood indicators of Nile tilapia fingerlings *Oreochromis niloticus*. Two levels of feed additives 0% and 1% Lactic acid with two feeding systems—1) half of the daily meal was offered in the morning and the other half in the afternoon and 2—) 3/4 (75 %) of the daily meal was offered in the morning and the other 1/4 (25%) in the afternoon. Fingerlings were stocked in 12 plastic aquaria (60 liters), with 10 fish/ aquaria. A commercial diet of 30% crude protein was used with a feeding rate of 3% of the biomass. The results showed, dietary LA had a positive impact on fish performance compared with feeding regimes. Fishes that were fed dietary LA with 50% of daily meal in the morning were the best in growth and blood parameters followed by fishes that were fed a diet without LA with 75% of their meal in the morning.

Key word: Nile tilapia, Lactic acid, growth performance, blood parameters, feeding regime.
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1. INTRODUCTION

Nile tilapia is essentially the only tilapia species that is cultured. It is the most cultured freshwater species among farmed tilapia, contributing ~71% of global total tilapia production and 81% of Egyptian aquaculture production (GAFRD, 2018). However, Egyptian aquaculture suffers from increasing production costs; feed cost as high as ~50% of the total production cost (El-Sayed, 1999). Therefore, increasing utilization of feed and improving its management can help reduce production costs. Organic acids are one of the tools used to improve feed efficiency in poultry (Bonetti *et al.*, 2020). They have been used to improve livestock performance and health as a potential substitute for antibiotic growth promoters. The use of dietary organic acids in aquatic animal culture has recently been the focus of much research and commercial interest. Organic acids are weak acids that contain one or more carboxylic groups (–COOH) and are produced by the microbial fermentation of carbohydrates by many species of bacteria during different metabolic processes (Ng and Koh, 2017).

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The most commonly used dietary organic acids in aquaculture for acidification are formic, acetic, propionic, lactic, and citric. Dietary acidifiers improve growth performance and nutrient availability in various aquatic species by increasing the secretion of digestive enzymes (Hossain et al., 2007). Moreover, they can reduce harmful microorganisms and promote beneficial microflora colonization of the gastrointestinal tract (Agouz *et al.,* 2015 and Soltan *et al.,* 2017). Feeding management also plays a great role in reducing feed costs by avoiding overfeeding, which reduces the amount of uneaten feed, thereby maintaining water quality for as long as possible. Feeding fish at the optimal time of the day and proper frequencies may optimize growth and feed efficiency and reduce waste (Abdel-Aziz *et al.,* 2016, 2020). This study aimed to evaluate the effect of lactic acid (LA) as a feed additive with different feeding strategies to increase the growth rate and improve feed utilization by Nile tilapia.

2. Materials and methods

2.1. Ethics statement

All experimental procedures, management conditions, handling, and sampling were approved by the Institutional Animal Care and Use Committee Research Ethics Board,

2.2. Site of work

The present experiment was conducted for 42 days at the Fish Feeding Laboratory, Faculty of Aquaculture and Marine Fisheries, Arish University, from May to June in 2021. Nile tilapia fingerlings were obtained from the Fish Research Center, Arish University, North Sinai, Egypt.

2.3. Work design and rearing conditions

This study was designed to evaluate two levels of feed additives 0% and 1% LA with two feeding systems—1) half of the daily meal was provided in the morning 9 a.m. and the other half in the afternoon 4 a.m. and 2) 3/4 (75 %) of the daily meal was provided in the morning and the other 1/4 (25%) in the afternoon. The treatments were formulated and named as shown in Table 1. Each treatment was in three replicates (Table, 1).

Table (1) experimental design

Groups	Feeding systems	lactic acid level
G1	1/2 of daily meal amount in the morning and the other in the afternoon	1%
G2		0%
G3	3/4 of daily meal amount in the morning and the other in the afternoon	1%
G4		0%

To carry out this work, 12 clear, square plastic aquariums (60 liters) were used and each aquarium was stocked with 10 fingerlings. All the fish were weighed individually immediately prior to the start of the trial to obtain the information about the initial biomass in each tank. The initial weight of fingerlings ranged between 17.14 to 18.33 g. The fish were kept in fresh water, and a 20% water exchange was carried out daily with a plastic pipette using the siphon method.

2.4. Feed and feeding

A floating commercial diet with a pellet size of 2 mm was used and contained 30% crude protein with gross energy of 19.00 kj/g diet (Table 2). Lactic acid (LA) was obtained from Finar Co.with molecular formula $C_3H_6O_3$ and molecular mass of 90.08 g/mol. LA was used as a feed additive at a rate of 10 ml/kg of diet and was mixed well; diets with or without LA were stored at 10ºC in the refrigerator. Fish were fed twice according to the experimental design at 9 am and 4 pm with a feeding rate of 3% of the biomass. The feeding rate was adjusted every 2 weeks according to the increase in weight of the fishes.

2.5. Water quality

Water temperature, pH, and dissolved oxygen were measured during the trial and were recorded every day at 1 pm. These parameters were estimated by a multipara meter water quality analyzer (MULP-8C), and ammonia nitrogen was analyzed every 2 weeks using chemical methods (APHA, 1992)

Table (2): The chemical analysis of the used diet (% Dry matter basis).

Items	%
Dry matter	95.50
Crude protein	30.00
Crude Fat	05.70
Crude fiber	04.38
Ash	08.72
NFE	51.34
Gross energy Kj/g*	19.00

Notice: - Chemical analysis was determined according to (A.O.A.C, 2010) .
*, was calculated according to (NRC, 1993)

2.6. Growth and feed utilization
Were calculated as follows:

- Weight gain (WG, g)
  FW-IW, where FW is the final weight and IW is the initial weight
- Average daily gain (ADG, g)=
  WG/t, where t is days
- Relative growth rate (RGR, %)=
  (WG/IW) × 100
- Specific growth rate (SGR, %)=
  (ln FW-ln IW/t) × 100
- Condition factor (K, %)=
  (FW/Fl^3) × 100, where Fl is the final length
- Survival rate (SR, %)=
  (Number of fish at the end/number of fish at the beginning of trial period) × 100
- Feed conversion ratio (FCR)=
  Feed intake, g per fish/WG, g
- Protein Efficiency Ratio (PER)=
  WG/protein intake
- Energy efficiency ratio (EER)=
  WG/energy intake

2.7. Sampling and chemical analysis

At the end of the experiment, 3 fish from each replicate were sampled at random and anaesthetized with t-amyl alcohol and killed with a cephalic blow, for conducting the chemical analysis of whole fish body (El-Haroun *et al.,* 2006). Compositions of feed and fish body were evaluated according to the methods described in AOAC (2000). Moisture content of the samples were assessed by drying in an oven at 105 ^{0}C for 24 hours; crude protein content CP (N×6.25) by the Kjeldahl method, ether extraction (EE) in a soxhlet system SOCS, ash by incineration in a muffle furnace at 600 ^{0}C for 6 hours and crude fiber (CF) by filter crucible and hot extractor unit with 150 ml 0.128 H_2SO_4 at 230 ^{0}C. Nitrogen free extraction content (NFE) was accounted as total carbohydrate by difference through, the following equivalent:-100-(protein +fiber+ fat+ ash) on basis dray matter. Gross energy for formulated diets was estimated with the factors 23.62, 39.5, and 17.56 kJ/g for CP, EE, and total carbohydrates, respectively (NRC, 1993)

2.8. Examination of blood

At the end of experimental period, Four fish from each replicate tank were anaesthetized using clove (50µl/l) samples of blood were drawn by 3-ml syringes from the caudal vein and emptied in two tubes one of them contained EDTA to prevent coagulation and estimate hematological parameters and other tube did not contain EDTA to measure the serum parameters .Thereafter, the samples tubes were immediately transported to hematological laboratory. White blood cells, leucocytes WBC; 10^9/l, red blood cells erythrocytes RBC; 10^{12} /l and hemoglobin, HB g\dl) were determined according to the standard methods as described by (Martins *et al.,* 2008 and Rawling *et al.,* 2009). Biochemical blood indicators such as plasma glucose (RBs, mg/d), aspartate aminotransferase (AST, U/l) and alanine aminotransferase (ALT, U/l), urea, mg/dl and createnine, mg/dl) were measured spectrophotometerically using commercial kids with a semi-automated analyzer (3000 Evolution), Bio-chemical, system, Int. Arezzo, Italy.

2.9. Statistical analysis

Data were analyzed via two-way analysis of variance. The differences among groups were determined via Wallar-Duncan at a significant level ($P \leq 0.05$) using SPSS Statistical Package Program version19, released version.

3. RESULTS

Table 3 shows a statistical description of some physicochemical parameters. The temperature was between 26.5℃ and 31℃, with an average of 28.82°C ± 0.32℃. The pH was between 7 and 8.8, with a mean of 8.09 ± 0.16. DO values were between 5.4 and 8 mg/l, with a mean of 6.45 ± 0.22 mg/l. Total ammonia nitrogen was between 0.01 mg/l and 0.37 mg/l, with average of 0.15 ± 0.01 mg/l.

Table (3) Means values of water quality measurements for all treatments.

Items	Range	Min	Max	Mean	SE	Variance
Temperature	4.50	26.5	31.0	28.82	0.32	1.037
pH	1.80	7.00	8.80	8.09	0.16	0.37
DO, mg/l	2.60	5.40	8.00	6.45	0.22	0.67
TAN, mg/l	0.36	0.01	0.37	0.15	0.03	0.015

3.1. Effect of dietary lactic acid on growth and feed efficiency of Nile tilapia fingerlings.

As shown in Table 4, growth parameters such as FW, WG, ADG, SGR were significantly influenced by adding LA to the tilapia diet. Tilapia fingerlings that were fed a diet containing 1% LA were higher in FW (29.59, g), WG (11.86, g), ADG (0.285, g), and SGR (1.22, %/day) than those fed a diet without LA. Other growth indicators like RGR, K, and FL were insignificantly different, but dietary LA had the highest. Likewise, feed utilization parameters did not vary significantly ($P \leq 0.05$) between the groups provided meals with and without dietary LA. However, fingerlings that were fed dietary LA had higher FI (15.40, g/fish), EER (0.212), and best FCR (1.59) than those fed an LA-free diet, which recorded the aforementioned parameters as 14, 25 g/fish, 0.159, and 2.11, respectively.

3.2. Effect of feeding system alone on growth and feed efficiency of Nile tilapia fingerlings.

Table 5 shown insignificant differences in all growth and feed utilization parameters between fingerlings that were fed half of their daily meal in the morning and the other half in the afternoon compared with those fed 3/4 of their meal in the morning and the other 1/3 in the afternoon. However, tilapia fingerlings fed that were half of their meal in the morning were better in growth and feed utilization than those fed 75% of their meal in the morning.

Table (4): effect of adding lactic acid (LA) as feed additive on growth and feed utilization of Nile tilapia fingerlings.

Items	Lactic acids as feed additive		Sig.	F-value
	1%	0.%		
IW, g	17.73±1.06	17.92±2.34	0.73	0.124
FW, g	29.59±0.55[a]	25.91±0.75[b]	0.001	19.77
WG, g	11.86±1.88[a]	7.92±0.86[b]	0.05	3.54
ADG, g/day	0.285±0.04[a]	0.188±0.02[b]	0.04	3.64
RGR, %	66.89±20.23	44.19±10.94	0.23	1.59
SGR, %/day	1.22±0.26[a]	0.87±0.16[b]	0.05	1.35
K, %	2.05±0.09	1.76±0.32	0.11	3.02
SR, %	100	100	-	-
FCR	1.74±0.38	2.11±0.47	0.40	0.75
PER	2.54±0.06	3.24±1.16	0.60	0.28
EER	0.212±0.05	0.159±0.03	0.41	0.74

Values are mean ± SD of triplicate groups in the same row with different superscripts (a,b) are significantly different ($P \leq 0.05$).

Table (5): effect of feeding system alone on growth and feed utilization parameters of Nile tilapia fingerlings

Items	Feeding strategy		Sig.	F-value
	1/2 of daily meal in the morning and other in afternoon	3/4 of daily meal in the morning and other in afternoon		
IW, g	17.56±1.84	18.05±1.48	0.86	0.031
FW, g	28.67±0.95	26.75±1.03	0.10	3.15
WG, g	11.20±2.24	8.70±0.51	0.31	1.11
ADG, g/day	0.267±0.05	0.206±0.012	0.30	1.18
RGR, %	63.78±22.06	48.20±9.06	0.39	0.79
SGR, %/day	1.16±0.29	0.93±.133	0.50	0.47
K, %	1.97±0.07	1.83±0.16	0.45	0.61
SR, %	100	100	-	-
FCR	2.08±0.55	1.78±0.30	0.82	0.055
PER	3.77±1.14	2.023±0.37	0.18	2.09
EER	0.202±0.054	0.168±0.03	0.60	0.29

Values are mean ± SD of triplicate groups in the same row

3.3. Interaction between dietary lactic acid and feeding system on growth and feed efficiency of Nile tilapia fingerlings.

There was a significant interaction effect of adding LA and different feeding systems on all growth and feed efficiency parameters, and this is illustrated in Table 6. Fish of G1 was highest in WG (16.02 g), ADG (0.388 g), RGR (93.47%), SGR, (1.57%/ day), FCR (0.75), and EER

(0.323), followed by G4, which recorded 9.67 g, 0.229 g, 54.23%, 1.03%/day, 1.13, and 0.237, respectively. WG, ADG, RGR, SGR, FCR, and EER did not significantly change between G2 and G3. There were no significant differences in PER and SR, which was 100% for all treatments. G1, G2, and G3 did not vary significantly in K, and they were significantly higher than G4 in K (1.50%).

Table (6): Effect of interaction between dietary lactic acid with different feeding system on growth and feed utilization efficiency of Nile tilapia fingerlings.

Items	Treatments				Sig.	F-value
	G1	G2	G3	G4		
IW, g	17.14±0.70[a]	18.16±3.60[a]	18.33±1.05[a]	17.83±1.99[a]	0.120	3.97
FW, g	33.19±0.31[a]	24.33±0.88[b]	26±0.50[b]	27.5±0.28[c]	0.001	26.29
WG, g	16.02±0.33[a]	6.17±0.88[c]	7.67±0.50[b]	9.67±0.28[b]	0.001	61.55
ADG, g/day	0.388±0.011[a]	0.146±0.020[c]	0.182±0.112[c]	0.229±0.006[b]	0.011	59.50
RGR, %	93.47±2.74[a]	33.99±4.85[c]	41.84±2.72[c]	54.23±2.43[b]	0.002	181.18
SGR, %/day	1.57±0.026[a]	0.690±0.085[c]	0.830±0.046[c]	1.03±0.030[b]	0.001	130.16
K, %	1.93±0.063[a]	2.01±0.148[a]	2.16±0.170[a]	1.50±0.037[b]	0.025	05.61
SR, %	100	100	100	100	-	-
FCR	1.075±0.016[b]	3.10±0.370[a]	2.42±0.180[a]	1.13±0.105[b]	0.001	25.90
PER	3.89±0.086	3.64±2.56	1.20±0.76	2.84±0.08	0.484	0.89
EER	0.323±0.006[a]	0.081±0.011[c]	0.100±0.006[c]	0.237±0.006[b]	0.001	195.46

Values are mean ± SD of triplicate groups in the same row with different superscripts (a,b.c) are significantly different ($P \leq 0.05$).

3.4. Effect of dietary lactic acid and different feeding systems on the whole-body composition of Nile tilapia fingerlings.

As shown in Table 7, there were significant differences between the groups for crude protein, fat, and ash. The highest body content of protein was achieved by G3 and G1, followed by G2 and G4. Although G4 had the highest content of body fat, followed by G2, they were significantly higher than G3 and G1. Regarding ash content, G3 had the lowest ash content G4 was the highest, whereas it did not vary significantly between G1 and G2.

Table (7): Effect of dietary LA supplementation with different feeding system on whole body composition of Nile tilapia fingerlings (on basis Dry matter)

Items	Treatments				Sig.	F-value
	G1	G2	G3	G4		
CP, %	58.75 ±0.75[a]	56.50 ±0.50[ab]	60.00 ±1.00[a]	53.50 ±1.50[b]	0.04	8.01
EE. %	*28.65 ±0.25*	*29.75 ±0.35*	*29.00 ±1.41*	*31.25 ±0.36*	*0.09*	*4.48*
Ash, %	12.33 ±0.42[b]	13.00 ±0.28[b]	10.60 ±0.42[c]	15.00 ±0.56[a]	0.00 3	34.90

Values are mean ± SD of triplicate groups in the same row with different superscripts (a,b.c) are significantly different ($P \leq 0.05$).

3.5. Hematological and Biochemical blood indicators:

Data in Table 8, showed that, there were significant differences among the treatments in some parameters of hematological indicators such as WBC and HB. Fish in G2 had the highest WBC followed by G3 and G4 while G1 was the least. The highest values of HB were 6.50, 5.4 g/dl which were recorded by G2 and G3 while G1 and G4 recorded 3.9 and 4.43 g/dl respectively. RBC did not significantly affect by treatments and recorded 2.3, 2.3, 2.6 and 2.1 ($\times 10^{12}$/l) for G1, G2, G3 and G4 respectively. In relation to biochemical blood parameters, RBs and AST were significantly differed among groups at level $p \leq 0.05$. RBs, mg/d did not significantly between G2 (81) and G3 (79) and they were significantly higher than G1 (72) and G4 (75).

Table (8): Effect of dietary LA supplementation with different feeding system on blood indicators of Nile tilapia fingerlings (on basis Dry matter)

Items	Treatments				Sig.
	G1	G2	G3	G4	
WBC (10^9/l)	111.73^b	176.20^a	134.40^{ab}	130.21^{ab}	0.020
RBC (10^{12}/l)	2.30	2.30	2.6.00	2.10	0.500
HB (g/dl)	3.90^b	6.50^a	5.400^a	4.43^{ab}	0.040
RBs (mg/d)	72.00^b	81.00^a	79.00^a	75.00^b	0.030
AST (U/l)	112.60^b	128.30^a	102.67^b	76.60^c	0.005
ALT (U/l)	31.33	35.66	35.00	34.30	0.970
Urea (mg/dl)	11.26	15.00	15.60	15.60	0.100
Creatinine (mg/dl)	0.92	0.97.00	0.94	0.91	0.440

Values are means of triplicate groups in the same row with different superscripts (a,b.c) are significantly different (P≤0.05).

G4 had the lowest AST (76.6, U/l) followed by G3 (102.67, U/l), G1 (112.6, U/l) and G2 had the highest value (128.30 U/l). There were insignificant changes among treatments in ALT, Urea and Creatinine however, G1 and G4 were the lowest in ALT and Creatinine compared with G3 and G2 was the highest in these indicators. Also G1 achieved the lowest value of Urea (11.26, mg/dl) compared with the other groups which recorded 15, 15.6 and 15.6, mg/dl for G2, G3 and G4 respectively.

4.DISCUSSION

Mean values of water physicochemical parameters during the experimental period were within the recommended values for aquaculture growth and tilapia culture according to Stone and Thomforde (2004), El-Sayed (2006), and Abdel-Aziz *et al.* (2019). Growth rate indicators, such as FW, ADG, and SGR, were significantly improved for fishes that were fed a diet containing 1% LA compared with those fed a diet without LA. The other indicators of growth and FCR were similar between the groups; they did not vary significantly. These findings agree with many previous studies, such as Tung and Pettigrew (2006) who reported that adding organic acids in aquaculture feed improves the growth of cultured fish. Baruah *et al.* (2008) reported that adding organic acids to fish feed has many advantages that improve fish performance. Eid (2012) found a significant enhancement in the WG of Nile tilapia fed with dietary organic acid blend and organic salt blend compared with the control group. Upadhaya *et al.* (2014) reported that fishes that were fed 1%–1.2% dietary organic acid had significantly higher FW. Sobhy *et al.* (2018) exhibited that adding organic acids to animal feed improves FCR, yield, and economic value. Furthermore, Asriqah *et al.* (2019) highlighted that adding organic acids in fish diet has positive results on growth, but the condition factor has no influence. Organic acids in fish feed encourage increased feed utilization by reducing the negative effects of antimicrobial agents in the digestive tract, as reported by Anani and Nunoo, (2016) and Sobhy *et al.*, (2018). Organic acids in fish feed reduced pH in the stomach and foregut and increased the activity of digestive enzymes such as pepsin and protease enzymes, thereby improving the protein metabolism and increasing the availability of minerals. Similar results were reported by Sugiura *et al.* (1998), Vielma *et al.* (1999) and Baruah *et al.* (2008) an increase in apparent availability of Fe, P, Mg, Ca in rainbow trout, which were fed a diet containing acids that highly solubilized animal protein sources especially in the fish stomach. Organic acids supplemented feed increases the level of amylase in the fish gut, and thus the utilization efficiency of dietary carbohydrates is increased. In the same context, it was found that citric acid increased the amylase activity in fish liver and intestine by 30.7% and 29.4%, respectively (Li *et al.*, 2009). Moreover, acidic conditions created in the fish gut because of using organic acids cause short-chain fatty acids such as butyric, propionic acid to be produced by microbes in the gut, improving the metabolic rate, thereby enhancing the absorbed nutrients (Huda-Faujan *et al.*, 2010). Like any other organic acid, LA has no harmful effects on fish performance and is considered a safe organic acid. Adams (1988) affirmed that LA in the diet of *Tilapa zilli*, or alone stimulated and increased the consumed feed. LA in fish feed reduces the unwanted pathogenic microbes, lowers the risk of antibiotic resistance, and reduces the discharge of phosphorous and total ammonia into the water, thereby preventing aquatic pollution and improving rates of nutrient absorption (Mathew *et al.*, 1991; Baruah *et al.*, 2008).

Results from other studies conflict with our findings, such as the growth rate of Olive flounder was not positively affected by dietary supplements with organic acids (Katya *et al.*, 2018). There were no significant differences in growth performance between European sea bass juveniles fed with dietary butyrate and those fed a butyrate-free diet (Busti *et al.* 2020) . Moreover, Hossain *et al.* (2007) completely disagree with our result; they reported that the growth performance of fish that were fed a diet containing 1% LA did not improve; this indicates that the beneficial effects of organic acids supplements in fish feed depend on the species, feed ingredient, dietary protein source, water quality, rearing condition, dose, and type organ acid used. A high level of dietary organic acid negatively influences pH balance in the fish gut, causing a decrease in fish growth, as found by (Asriqah *et al.*, 2018). Increasing the amount of offered feed at the optimum time according to the fish behavior has a positive effect on feed utilization (Abdel-Aziz *et al*., 2016 and 2020). Bolliet *et al.* (2001) confirmed that feeding time affects fish growth. In contrast, several studies emphasized that feeding in the dark for *Heterobranchus longifilis* and *Clarias lazera* had a positive effect on SGR and FCR than those fed the same amount during the daylight (Kerdchuen, and Legender, 1991: Baras *et al.*, 1998). The opposite was observed in rainbow trout (Eriksson *et al*., 1993). Moreover, rainbow trout that were fed at dawn achieved better growth than those that were fed at midnight (Gélineau *et al.*, 1996). Therefore, the increase in offered feed at the optimal time is scientifically investigated to improve feed utilization, growth rate, and economic efficiency. Gélineau *et al.* (1996) found that protein and lipid are most efficient in rainbow trout that are fed at dawn. Therefore, the amount of feed delivered with each meal should be altered according to the normal daily pattern of feeding activity; it may potentially improve fish growth. This was supported by Azzaydi *et al.* (1999) who reported that sea bass fed three meals daily, with amounts adjusted following the rhythm of self-feeding fish, had greater WG than those fed three equally sized-meals daily. Regarding tilapia fish, Abdel-Aziz *et al.* (2020) confirmed that feeding tilapia in the morning had a positive effect on growth rate compared with in the afternoon. Tilapia fed 2/3 of their daily meal recorded higher WG and SGR than those fed 2/3 of their meal in the afternoon (Abdel-Aziz et al., 2021). The opposite trend in various studies was observed on carp, sea bass, and channel catfish that feeding time did not affect fish performance (Robinson *et al.* 1995; Boujard *et al.* 1996). Our results showed that increasing the offered feed from 50% to 75% in the morning did not significantly affect growth or feed efficiency parameters. This may be attributed to adding 1% of LA in tilapia feed, which was very commensurate with feeding an equal amount twice, but increasing the offered feed for fish in the morning led to an increase in the level of LA, negatively affecting growth and feed parameters. Thus, the growth and feed indicators for fish fed 75% of their daily meal did not differ from those fed 50%. Although fishes that were fed dietary LA with 50% of their daily meal in the morning (G1) had the highest growth performance and the best FCR, followed by fishes that were fed a diet without LA with 75% of their daily meal (G4). Despite the insignificant differences between G2 and G3, G3 was better in SGR and FCR than G2, which was fed a diet without LA with half of their daily meal in the morning. This confirmed that LA at 1% as a feed additive is more effective when fish are fed half of their daily meal in the morning compared with depending on an increase of the offered feed at the optimum feeding time of fish. FCR of G1 reached the best rate; moreover, this group had the best PER and EER. These results are in accordance with those of Ringo *et al.* (1994) who found that the 1% Na lactate diet improved FCR. Sugiura *et al.* (1998) mixed the feed pellet with organic acid for storage improves FCR; furthermore, Sobhy *et al.* (2018) concluded that dietary lactic acid supplemented significantly improved FCR and PER of Nile tilapia in comparison with the control group. Ramli *et al.* (2005) reported that adding formic acid with 0.2% to 0.5% improved PER. However, Asriquh *et al.* (2018) revealed that dietary organic acids had an insignificant effect on FCR and SR; this statement completely conflicts with our findings. Complementing the above, adding organic acid at the optimum dose in the fish diet improves the anti-inflammatory response to the intestines, thereby protecting the epithelial integrity; dietary organic acid also increases the development of beneficial bacteria like *Lactobacillus* and *Bacillus sp.* in the fish gut. G4 had better FCR, PER, and EER than G3 and G2, respectively; this may be due to maximizing utilization of feeding of tilapia fish in the morning by adding a larger amount of daily meal during the feeding time. Abdel-Aziz *et al.* (2016) confirmed that feed utilization of rabbitfish fry was improved by morning feeding. Moreover, there is a linear relationship between the optimum feeding time and protein metabolism rate, with nutrient retention then decreasing ammonia excretion in the rearing water. Furthermore, the physiological case of fish reflects the optimum feeding time, so that fish fed in the morning or the photo phase had significantly lower plasma insulin than those fed in the afternoon or the evening (Bolliet, 2000). Accordingly, tilapia fish are offered a higher amount of feed in the morning to maximize feed utilization.

The results of fish body composition agreed with Agouze *et al.* (2015) who indicated that organic acids in tilapia feed significantly affect fish body composition. They found that when fish were fed a diet containing organic acid, protein content increased and fat content decreased. Moreover, Noeske-Hallin *et al.* (1985) observed the optimum feeding system increased WG and fat deposition of channel catfish; this was observed with fish of G4. A similar result was obtained by Sobhy *et al.* (2018) who concluded a negative relationship between body content of CP and EE when LA was used in tilapia feed. Our result did not agree with that of Vielma *et al.* (1991), who found that the ash content in the body increased in fishes that were fed dietary acids. Baruah *et al.* (2008) confirmed that the body ash content of juveniles of *Labeo rohita* was not significantly affected by citric acid.

As it is known blood indicators reflect the fish health, immunological status and use to assess the diets quality and their additives as reported by (Hari *et al.*, 2004). In the light of the results of Table (8) it can be noted that physiological status improved with G1 and G4 compared to G3 while the physiological statues of G2 was the least. Decreasing WBC count indicates that fish in the best health status, where in WBC increases upon stressful conditions as reported by (Harikrishnan *et al.*, 2010) also, may be influence on gut polyamine production an anti-stress substance which also reduces WBC count in fish blood as confirmed by (O'Conor *et al.*, 1986) HB increases in fish as a result of exposure to suboptimal conditions or stressful with no feeling of comfort then the demand of oxygen increases causing the erythrocytes to synthesiz more HB in order to maintain the optimum oxygen level in blood (Desimira Dicu et al., 2013) So G2 had the highest value of RBC and (Montero *et al.,* 1999) affirmed that the improper rearing conditions have been caused a significant increase in RBC These results were at variance with study of Sobhy *et al.,* (2018) who found that, WBC, RBC and HB of fish fed dietary LA enhanced significantly compared with those fed dietary without LA. And other study of Soltan *et al.,* (2017) who using the organic acids at different concentrations in fish diet lead to increase RBC and WBC. On the other hand, a similar trend with our work was reported by Abdel-Aziz *et al.* (2020) who administrated that fish fed with 2/3 of their daily meal in the morning had the lowest count of WBC, RBC and HB. Regarding the serum parameters, the decreased levels of RBs, AST, ALT, Urea and createnine indicate to improve the health or immunological status and fish welfare. a high level of RBs in fish blood reflects a higher stress status of fish. In the same Mohapatra *et al.* (2014) said that, increasing the gluconeogenesis with fish that were reared under stressful conditions resulting an increase in RBs level. Our study showed that the lowest level of RBs was obtained with G1 and G4 then G3 while G2 had the highest level. The evaluation of serum enzyme activities of AST and ALT are considered as an indication of the amount of liver damage or hepatocellular damage (Barraze *et al.,* 1991). Increasing the AST and ALT clear a distortion and harmful in liver. Our study showed that fish fed (G4, G3) 75% of their daily meal in the morning had lower level of AST than those fed (G1, G2) 50% of their meal in the morning. Moreover the G1 and G4 were lower in ALT than G2 and G3. Blood level of Urea and Creatinine can indicate whether the kidneys are functioning properly (Walke *et al.,* 1999). Ajeniyi and Solomon (2014) reported that, normal range of blood creatinine is from 0.8 to 1.4 mg/dl, this agrees with our results which revealed that createnin ranged between 0.91 to .97 mg/dl.

5.CONCLUSION

Fishes that were fed a diet containing 1% LA showed better growth performance than those fed a basal diet without LA. Moreover, there were no significant differences in feeding systems regardless of the addition of LA. The interaction between the tested factors (dietary LA and feeding system) was significantly higher for all parameters in fishes that were fed a diet containing 1% LA and given 50% of their meal in the morning, followed by fishes that were fed a diet without LA and given 75% of their meal in the morning and fishes that were fed a diet containing LA with 75% of their meal in the morning. Fishes that were fed a diet without LA with 50% of their meal in the morning had the lowest growth rate and FCR. Additionally, blood parameters improved in the same manner of growth parameters. Finally this study recommends that using 1% of LA as feed additives in tilapia feed improves the fish performance regardless the used feeding system but In the case of using a diet without LA it is preferable to feeding the fish with 75 % of their daily meal in the morning.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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