Impact of protected and non-protected lactic acid used as an acidifier in the diet on *Oreochromis niloticus*

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ARTICLE INFO

ABSTRACT

The present study was conducted to compare between protected and non-protected lactic acid on growth performance, feed utilization, some hematological parameters and carcass proximate analysis in *Oreochromis niloticus* (*O. niloticus*). Three iso-nitrogenous and iso-caloric diet contained (37.5% ± 0.97) crude protein (4427 cal/g ± 39) Gross Energy (GE) were formulated. The supplemented diet with 0.2% non-protected lactic acid (T1), 0.2 % protected lactic acid (T2) and diet with no additive (T3) were fed individually to three equal fish groups (25 fish/set up with an underlying body weight of 5.42 ± 0.07g) for 90 days. At the end of the feeding trial, *O. niloticus* offered the control diet exhibited lower growth and feed utilization rates than protected and non-protected lactic acid. Fish fed the diet T2 showed the highest final body weight (FBW), final weight gain (FWG), average daily gain (ADG), feed intake (FI) and survival rate (SR). Fish fed a diet (T2) showed improvement in the tested blood parameters compared to the control group. The present observations suggest that supplementation of lactic acid into the fish diet can be used as an acidifier for growth promoting purpose. In addition, protected lactic acid has a significant effect compared to the non-protected one.

INTRODUCTION

Fish is considered a valuable and affordable source of protein; which are reared in intensive aquaculture systems for obtaining a high yield. A wide range of diseases and consequent fish mortalities has been emerging worldwide due to high stocking density adopted in intensive systems. Several control approaches were used for combating these pathologies and their consequent mortalities. Using antibiotics for the disease treatment / prevention is neither effective nor consumer or environment-friendly. In particular, use of antibiotics in aquaculture has been extensively reproved and the application has already been confined, realizing their counteractive impacts not only on fish and human health but also on the aquatic environment (Katya *et al.,* 2018).

Organic acids (OA) are weak acids with at least one carboxylic group (–COOH) and a carbon chain having one to seven carbon atoms. Organic acids have been employed as a potential replacement of antibiotic growth promoters to improve the performance and the health of farm animal.

Formic, acetic, propionic, lactic and citric acid are the most commonly used dietary OA in aquaculture for acidifying purpose. Dietary acidifiers have demonstrated effective in enhancing the growth performance and the nutrient availabilities in various aquatic species as they reduce the pH of the digesta in the stomach and the foregut, which in turn stimulates the pepsin activity, improving protein digestibility and mineral absorption. Dietary inclusion of OA enhances the bioavailability of minerals, including phosphorus, magnesium, calcium, and iron (Soltan *et al.,* 2017).

The short-chain OA is mostly absorbed through the intestinal epithelia providing energy for renewing the intestinal epithelia and maintaining the gut health. Oral administration of OA significantly improves the feed intake, the live weight gain, the feed conversion ratio and the protein efficiency ratio of various Tilapia species. From another point of perspective, OA can improve the general health status by its antimicrobial effect against pathogenic bacteria. Due to the adverse effect of the gastric acids on the dietary OA which causes its dissociation before reaching the hindgut where it must come in contact with the pathogenic bacteria and where it must dissociate causing decrease of the pH making the surrounding environment unfit for the reproduction and/or survival of the pathogenic bacteria, development of coating technologies, protecting acids by matrix coating or encapsulation for targeted delivery to different gut segments was conducted. Protected OA are more effective in retarding absorption of dietary acids and allowing more effective delivery of the acids to the distal ileum, cecum, and colon (Nermeen *et al.,* 2015).

Therefore, the aim of this study was to make a comparison between the effect of non-protected and lab-developed protected lactic acid as a growth promoter in Nile tilapia.

MATERIALS AND METHODS

Experimental design:

One hundred fifty monosex (male) Nile tilapia fingerlings, *Oreochromis niloticus* with an average initial body weight of (5.42±0.07g) were obtained from a private farm at Fayoum governorate, Egypt. Fish were acclimated to the experimental conditions for two weeks (15 days) during-which fish were fed a control diet at a level of 3% of the biomass. Three treatments were tested in duplicates using six aquaria, which were stocked with 25 fish each. The different treatments are illustrated in (Table 1).

Table 1: Experimental design of the present study

Group	Treatment	Dosage (%/g diet)
T1	Non-protected lactic acid	0.2%
T2	Protected lactic acid	0.2%
T3	Control	-

Experimental Diet:

The control diet was iso-nitrogenous and iso-caloric diet contained (37.5% ± 0.97) crude protein (4427 cal/g ± 39) Gross Energy (GE) and was formulated to meet the nutrient requirements recommended by NRC, (1998) (Table 2).

Experimental system:

The present study has been carried out in the laboratory of Fish Nutrition, Regional Center for Food and Feed, Agricultural Research Center, Egypt from 18th August till 17th November 2017 (90 days). The experimental fish were fed twice a

day at a feeding rate of 3% of their live body weight. Fish in each aquarium were weighed every 15 days. The daily feed ration (DFR) was calculated using the average body weight (ABW), the total number of the fish (N) and the feeding rate per day (FRd^{-1}) were performed according to Nandlal and Pickering, (2004) using the following formula: DFR=ABW×N×FRd^{-1}. The number of dead fish were daily recorded and removed from experimental tanks. A photoperiod of 12-h light, 12-h dark (08:00–20:00 h) was used via fluorescent ceiling lights supplied the illumination.

Table 2: The formula of the basal diet (%) and chemical analysis (%) dry matter basis for Tilapia fish

Ingredients	%
Soybean meal (CP 46%)	63.3
Yellow Corn	13.3
DDGS	8.0
Wheat flour roughage	5.0
Limestone	2.3
Soybean seed High fat	2.1
Fish meal low fat (CP 65%)	2.0
Fish oil	1.6
Monocalcium phosphate	1.5
Salt (NaCl)	0.8
Vitamin and Mineral mixture [1]	0.1
Total	100
Chemical analysis %	
Dry matter	91.3
Crude protein	37.5
Crude lipid	3
Ash	7.8
Fiber content	3.68
NFE[2]	48.02
ME[3]	4427 (cal/g)

[1]Vitamin and mineral mix (mg / Kg diet): Vitamin A 10000000 IU, Vitamin D3 2000000 IU, Vitamin E 50000 mg, Vitamin K3 10000 mg, Vitamin B1 10000 mg, Vitamin B2 10000 mg, Vitamin B6 15000, Vitamin B12 20 mg, Niacin 50000 mg, Biotin 500 mg, Folic acid 50000 mg, Pantothenic acid 40000 mg, ZnO 50000 mg, MgO 25000 mg, $Fe_2(SO4)_3$ 75000 mg, CuSO4 15000 mg, Ca $(IO3)_2$ 3000 mg, Na_2O_4Se 300 mg, $CoCO_3$
[2]NFE (Nitrogen free extract) =100-(crude protein + lipid + ash +fibre content).
[3]Metabolizable energy (KJ g-1), calculated based on the physiological fuel values according to Brett, (1971).

Water quality:

Water temperature, dissolved oxygen, pH and total ammonia were monitored and adjusted during the study to maintain water quality at an optimal range required for Nile tilapia. Total ammonia was measured three times a week according to (APHA, 1999). The water parameters had averaged (±SD): The water temperature (27.1±0.3°C), dissolved oxygen (5.6±0.8 mg/L), pH (7.5±0.3) and ammonia (0.01 mg/L).

Organic acids:

Preparation of protected organic acids:

The matrix was prepared according to Andera *et al.,* (2002) to which the used OA (lactic acid 85%; Sigma, Aldrich) was added in a ratio 60:40 to obtain a final concentration of 50% lactic acid.

Preparation of non-protected organic acids:

Lactic acid 85% (Sigma, Aldrich) was added to distilled water (Milli-Q) with ratio 3:2 to obtain a final concentration of 50% lactic acid.

Preparation of lactic acid supplemented diet:

The required inclusion rate of lactic acid (0.2%) was added to the diet by gently spraying and mixing part by part. The lactic acid supplemented diets were packed in sterile polypropylene containers and stored at room temperature. Fresh diets were prepared bi-weekly to ensure the stability of the level of the acid all over the duration of the experiment.

Growth and feed utilization indices parameters:

Records of live body weight (BW/g) and standard body length (BL/cm) of fish were measured every 15 days during the experimental period. Growth performance parameters were calculated by using the following equations according to the methods described by De Silva and Anderson, (1995):

Condition factor (K):

$$K = (W/L3) \times 100$$

Where: W = weight of fish in grams and L = total length of fish in "cm"

Weight gain (WG, g) = Final weight (g) – Initial weight (g)

Specific growth rate (SGR % / day): was estimated using the following equation:

$$SGR = \frac{LnW2 - LnW1}{t} x \ 100$$

Where:

Ln = the natural log; W1 = first fish weight; W2 = the following fish weight in grams and   t = period in days.

Feed conversion ratio (FCR) was calculated by the equation:

$$FCR = \text{Feed ingested (g)/Weight gain (g)}$$

Relative food consumption (RFC) was measured by the following equation:

100 (food consumed (g) / 0.5 (final weight, g – initial weight, g) X time, days)

Protein efficiency ratio (PER) was measured by the following equation:

$$PER = \text{Weight gain (g)/Protein ingested (g)}$$

Survival rate: SR, % = (Z/X) × 100

Where: Z is the surviving fish number and X is the initial fish number.

Hematological and biochemical blood indices:

At the end of the experiment, blood samples were collected from the caudal vein of fish (Eissa, 2016) in all treatments, which then has been divided into two portions. The first portion was collected with anticoagulant 10% ethylene diamine tetraacetate (EDTA) to determine the hematocrit (Htc) and hemoglobin (Hb) according to the standard methods as described by Rawling *et al.,* (2009). The second portion of the blood samples was allowed to clot overnight at 4°C and then was centrifuged at 3,000 rpm for 10 min. The non-hemolysed serum was collected and stored at −20°C until use. Levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated according to the method described by Reitman and Frankel, (1957).

Proximate analysis of fish and experimental diets:

At the end of the experiment, three fish were chosen randomly, and the proximate analysis of whole fish body was performed according to AOAC, (2006). Fish and diet samples were oven dried at 105ºC for 24 h, ground, and stored at −20ºC for subsequent analysis. Dry matter was determined after drying the samples in an oven at 105ºC for 24 h. Ash was determined by incineration at 550ºC for 12 h. Crude

protein was determined by micro-Kjeldhal method, N × 6.25 and crude fat by Soxhlet extraction with diethyl ether (40ºC–60ºC). The crude fiber content of diets was determined by mixing ground sample (2 g) with sulphuric acid (200 ml 1.25% W/V). The mixture was boiled under a reflux condenser for 30 minutes, followed by filtration through Gooch crucible provided with the asbestos mat. The nitrogen-free extract was computed by summing the values of crude protein, crude lipid, crude fiber and ash, and then subtracting this sum from 100.

Statistical analysis:

All data were analyzed by using the software SAS, version 9.1 (SAS, 2004). Differences between means were tested by Duncan, (1955) new multiple range test. GLM procedure was used for analyzing the effects of lactic acid (protected/non-protected). All differences were considered significant at P≤0.05 and the results are presented as means with a pooled standard error of the mean.

RESULTS AND DISCUSSION

A potential concern of fish culturists is reduced resistance to bacterial and viral infections, which could be caused by inadequate rearing conditions and/or malnutrition. The preventative effects of supplements on disease resistance have been investigated by examining growth performance, mortality and blood properties (Nakagawa *et al.*, 1987). Routine use of antibiotics as growth promoters is a matter of debate in the animal farming industry, in the field of aquaculture it is well established so far that the inclusion of antibiotics into the diets of fish (Ahmad and Matty, 1989) can promote growth and feed conversion. However, the use of low levels of these antibiotics in animal feeds possesses the possibility to transfer bacterial immunity to species pathogenic in animals and humans (Liem, 2016). Gram-negative bacteria affected all type of fish of either marine or freshwater fish all over the world in the different areas of Asia, America, Australia, Africa and Europe, so it was crucial to find out safe alternatives to be used as precautionary approach (Austin and Austin, 1999).

Using acidifiers in the fish diet is considered as one of the most effective approaches not only to control colonization of pathogenic bacteria in/on the animal body but also to improve growth performance, increase survival rate and positively affects the chemical composition of the products of livestock including fish (Luckstadt, 2008). The efficiency of an OA to inhibit the growth of a microorganism depends on its pKa value, which describes the pH value at which the acid is available in its dissociated and un-dissociated form respectively. Alone in its un-dissociated form, the OA has its antimicrobial power as they can reach through the walls of bacteria and fungi and alter their metabolism. Accordingly, OA with a high pKa value are weaker acids and therefore more effective preservatives for feed, as, being present in the feedstuff with a higher proportion of their un-dissociated form and can protect feed from fungi and microbes (Ettle and Roth, 2005).Therefore, the lower the pKa of the OA (the higher proportion of dissociated form) the greater is its effect on the reduction of stomach pH and the lower its antimicrobial effect in the more distal portions during its transit through the digestive tract. A strong acid (with low pKa) will acidify the feed and the stomach, but will not have strong direct effects on the microflora in the intestine.

Protecting the OA by encapsulation or matrix coating ensures the arrival of it to the intestine in a non-dissociated form (Ravindran and Kornegay, 1993). However, the effectiveness of feeding acids varies with the types of the acid, the health status of

the animal and feed characteristics (Blank *et al.,* 1999 and Mroz *et al.,* 2006). Protected OA used in the current study are coated with a lipid base. The supplementation of diet with OA has been reported to improve growth performance by reducing gastrointestinal pH and subsequent modification of the intestinal microflora and performance parameters (Kirchgessner and Roth, 1982).

Growth performance of *Nile tilapia* as affected by non-protected and protected lactic acid:

It is clear from the obtained data in Table (3) that, there was no significant difference between all body weight values at the beginning of the experiment and by the effect of different treatments under this study; final body weight at the end of the duration was increased significantly in group T2, which was fed 0.2% protected lactic acid followed by group T1 which fed 0.2% non-protected lactic acid and the values of T1 and T2 were significantly higher than that obtained in the control group.

Table 3: Effect of lactic acid (protected and non-protected) on growth performance and survival rate of Nile tilapia *O. niloticus*.

Treat.[*]	IBW	FBW	IBL	FBL	FWG	IK	FK	ADG (g)	SGR (%/d)	SR
T1	5.46 [a]	30.00 [b]	6.78 [a]	11.72 [a]	24.54 [b]	1.77 [a]	1.87 [a]	0.27 [b]	5.49 [a]	92.0 [b]
T2	5.44 [a]	30.77 [a]	6.87 [a]	11.63 [a]	25.33 [a]	1.70 [a]	1.96 [a]	0.28 [a]	5.29 [ab]	100.0 [a]
T3	5.46 [a]	25.24 [c]	6.97 [a]	11.09 [b]	19.79 [c]	1.62 [a]	1.85 [a]	0.22 [c]	4.59 [b]	88.0 [c]
SE	0.00	0.03	0.09	0.04	0.03	0.07	0.03	0.00	0.15	0.00

[a-c] Means with the same letters within each column of the trait are non-significantly different (P≥0.05)
[*] Treatments as described in Table 1.

This result agrees with that obtained by Ricke, (2003); Upadhaya *et al.,* (2014a); Upadhaya *et al., (*2014b) who recorded significantly higher final body weight of farm animals fed diet supplemented with different concentration of OA ranged from 0.1% to 1.2% in the final diet. Other studies have also supported these findings (Tilman and Eckel, 1998; Tung and Pettigrew, 2003) which reported that supplementation of OA to aquaculture diet caused improvement of growth performance parameters including final body weight.

Data from the same table showed that, while there was no significant difference in the initial body length; a significant difference was recorded at the end of the experiment between the groups fed the OA treated diet compared to the control group with the T2 group has a significant difference body length than the shorter T1 group. These findings were supported by that obtained by (Ramli *et al.,* 2005) who found a significant increase in the length measurement of aquaculture treated with OA compared to the control group.

Many studies (Tilman and Eckel, 1998; Tung and Pettigrew, 2003; Jia *et al.,* 2010) supported the results of average daily gain (ADG), specific growth rate (SGR) and survival rate (SR) which were significantly higher in T2 group compared to T1 and T3 with the values of T1 are significantly higher than that of T3 and these studies concluded that, using acidifiers (0.2, 0.3 or 0.5%) significantly (P<0.05) improved BW and WG of *O. niloticus* (Ramli *et al.,* 2005). In a recent study, the supplem-entation of the basal diet by 1% Ca-lactate significantly (P<0.001) improved BW, BL, WG and SGR of Nile tilapia, *O. niloticus* (Mogheth, 2012). In another study, the OA significantly enhanced WG and SGR of *O. niloticus* compared to control group (Eid, 2012). For other fish species, (De Wet, 2005) described that final BW and SGR of rainbow trout fingerlings, *Oncorhynchus mykiss* were significantly improved with increasing acid blend inclusion (from 0.5 to 1.0 or 1.5%) versus control (P<0.05). Arctic charr, *Salvelinus alpinus* fed the diets supplemented by each of 1% Na-lactate

significantly (P<0.05) improved SGR (Ringø, 1991; Gislason *et al.,* 1996). Also, another study found that supplementation of Arctic charr diets by 1% OA significantly (P<0.05) improved SGR compared with control fish group (Ringø *et al.,* 1994).

Feeding aquaculture, including fish with OA, supplemented diet is clear to have a growth promoting and protecting effect, which was clear from the obtained data in the present study. Increasing in growth performance parameters like body weight, body length, daily gain, and growth rate, indicated that, OA could affect positively the physiological activity of fish body causing augmentation of feed utilization and consumption of its nutrient content. The similarity in the K factor in all groups indicates that lactic acid has no harmful effect on body parameters in the used concentration and considered as safe as the non-treated diet (Anani and Nunoo, 2016).

Feed utilization of *Nile tilapia* as affected by non-protected and protected lactic acid:

Data in Table (4) showed that; supplementation of OA in the fish diet caused a decrease in the RFC and FCR, which indicated the increase of the weight gain and decrease in the feed intake. The values of both parameters were significantly lower in T1and T2 compared to the value obtained in T3 with values of T1 recorded the lowest significant reduction.

Table 4: Effect of lactic acid (protected and non-protected) on feed utilization of Nile tilapia *O. niloticus*

Treat.[*]	FI	RFC	FCR	PER
T1	31.58 [b]	2.86 [c]	1.05 [c]	2.08 [a]
T2	34.14 [a]	3.00 [b]	1.11 [b]	1.98 [b]
T3	30.10 [c]	3.38 [a]	1.93 [a]	1.75 [c]
SE	0.03	0.007	0.003	0.003

[a-c] Means with the same letters within each column of the trait are non-significantly different (P≥0.05)
[*] Treatments as described in Table 1.

These results are supported by that of (Tilman and Eckel 1998; Tung and Pettigrew, 2003; Upadhaya *et al.,* 2014a; Upadhaya *et al.,* 2014b) who reported that the addition of OA in animal and aquaculture diet caused a reduction in the RCF and FCR indicating an improvement of the yield and economic value of the raised animal/aquaculture.

Protein efficiency ratio was shown to be significantly higher in T1 and T2 compared to T3 with the value of T1 significantly higher than that of T2. This result is supported by that obtained from previous studies, which improved that supplementation of animal diet; including aquaculture with OA increased the utilization of dietary protein (Tilman and Eckel, 1998; Tung and Pettigrew, 2003).

Using OA from day 1 to day 85, significantly (P<0.01) improved feed intake and the improvement was greater for 0.2 and 0.5% inclusion rate (Ramli *et al.,* 2005). Supplementation of tilapia diets by Ca-lactate significantly (P<0.05) increased feed intake (Mogheth, 2012).

Lactic acid has been reported to be effective in stimulating or enhancing feeding behavior when applied individually or together with other extractive compounds in *Tilapia zilli* (Adams, 1988).

Supplementation of the basal diets by OA including lactic acid significantly (P<0.05) improved FCR of *O. niloticus*. During 90 day experimental period FCR for *O. niloticus* fed the control diet showed the highest (worst) FCR compared to the

other experimental diets supplemented with the different doses (0.5, 1.0 or 1.5%) of either OA or its salts (Mogheth, 2012).

The improvement in growth performance and feed utilization due to acidification may be due to increasing the absorbance and availability of different minerals and increasing secretion of some enzymes such as proteases.

Effect of protected and non-protected lactic acid on proximate composition (% DM basis) of *Nile tilapia*:

Data obtained from the Table (5) indicated that; analysis of whole carcass composition revealed no significant difference in the dry matter content among the tested groups. Crude protein was shown to be higher significantly in T2 followed by T1 that were both higher than that obtained in T3. Fat content was found to be significantly higher in the control group compared to T1 and T2 with T2 showed significantly higher content than that in T1. Ash content is shown to be significantly higher in the T3 group compared to T1 and T2 with no significant difference between its value in T1 and T2. Therefore, a negative relationship was found between protein and fat content while no significant difference was detected between protected and non- protected OA.

These results are in agreement with those obtained by (Goda, 2002; Mogheth, 2012; Agouz *et al.,* 2015) who found a negative correlation between protein and fat content in whole carcass analysis and with those obtained by (Pandey and Satoh, 2014) who reported a higher content of ash in fish fed OA treated diet. Previous studies (Soltan *et al.,* 2017b) agreed with the obtained results in this study concerning the fat content as it revealed that fish fed OA supplemented diet has a significantly lower fat content than the control group.

Also, the increase of protein content in fish fed OA supplemented diet was clear from the data shown in the previous study. Dry matter of fish in all tested groups was similar with no significant differences in their values the result of which was supported by (Soltan *et al.,* 2017b) who reported the same trend.

Table 5: Effect of lactic acid (protected and non-protected) on proximate chemical analysis of Nile tilapia *O. niloticus*

Treat.[*]	Dry matter	Crude Protein	Fat	Ash
T1	25.6 [a]	58.2 [b]	20.2 [c]	11.7 [b]
T2	25.7 [a]	61.6 [a]	22.1 [b]	11.1 [b]
T3	26.4 [a]	54.7 [c]	25.3 [a]	14.9 [a]
SE	0.58	0.33	0.30	0.35

[a-c] Means with the same letters within each column of the trait are non-significantly different (P≥0.05)
[*] Treatments as described in Table 1.

Effect of protected and non-protected lactic acid on blood parameters of *Nile tilapia:*

The data in Table (6) showed the effect of OA supplementation on some blood parameters. It is clear from the obtain data that significant improvement of Hb and Htc occurred by the effect of OA if comparing the obtained values with those of the control group indicating a significant improvement in the general health status.

The decrease in liver enzymes is an indicator for the healthy liver as increasing these enzymes indicating destruction of liver tissue followed by liberation of these indicator enzymes. Results obtained in this study showed lower significant valued in liver enzymes compared to those obtained in the control group with fish in the T2 group has the best significant low values. These data were confirmed by those obtained by (Tilman and Eckel, 1998; Tung and Pettigrew, 2003; Adil *et al.,* 2010;

Soltan *et al.,* 2017 (a, b)) who calculated the supplementation of animal/fish diet with OA increased Hb and Htc and significantly reduced liver enzymes.

Table 6: Effect of lactic acid (protected and non-protected) on blood parameters of Nile tilapia *O. niloticus*

Treat.[*]	Hb (g/dl)	Htc (%)	RBC (10^6/mm^3)	WBC (106/mm3)	ALT (U/l)	AST (U/l)
T1	8.95 [a]	29.50 [a]	2.33 [c]	84.60 [a]	17.50 [b]	115.50 [b]
T2	7.75 [b]	25.65 [b]	2.59 [a]	76.05 [b]	15.00 [c]	94.00 [c]
T7	7.25 [c]	24.05 [c]	2.46 [b]	56.90 [c]	31.00 [a]	213.00 [a]
SE	0.14	0.39	0.01	0.14	0.48	0.45

[a-c] Means with the same letters within each column of the trait are non-significantly different (P≥0.05)
[*] Treatments as described in Table 1.

Data obtained from the same table indicated that the number of RBCs and WBCs increased significantly in T1 and T2 if compared to the values obtained in the control group indicating and assuring improvement in the health condition of the treated fish. These findings are in agreement with (Tung and Pettigrew, 2003; Sherif *et al.,* 2013; Soltan *et al.,* 2017 (a, b)) who found the addition of OA in the fish diet in different concentrations caused a significant increase in blood parameters including RBCs and WBCs.

Reduction of mortality rate (an increase of survival rate) indicates the protective effect of the supplemented OA as it elevates the general body condition, including blood parameters (RBCs, WBCs, Hb, Htc) which could protect its body against stress factors and pathogenic infection. In addition, decreasing the liver enzyme values indicates good health condition, assuring the protecting activity of lactic acid.

Having non-protected lactic acid with the best effect on Hb, Htc, RBCs and WBCs if compared with the values of protected lactic acid and the control group may be due to absorption of larger amounts of it before it reached the distal part of the gastrointestinal tract where its effect mainly lowering pH and interfering with the activity of pathogenic bacteria.

Also, due to the introduction of the non-protected acid into the gastrointestinal tract where there is low pH, part of lactic acid may undergo dissociation with the liberation of lactate which is an essential and crucial substance for energy supply to all body cells including blood cells causing increased blood cell number and consequently the Hb content (Lampe *et al.,* 2009).

Improving performance parameters is considered as one of the most important gains from using OA as feed supplements as it causes lowering of intestinal pH and acts as inhibitory environment to pathogenic bacteria which decrease the involvement of the immune system in consuming great part of the dietary proteins in formation of antibodies to attack the pathogenic bacteria allowing this protein to share in the formation of muscles. Therefore, increase the musculature of the living animal/fish and subsequently the weight gain and finally the final body weight is a definite result of using OA in this case.

Also, the presence of the acidic conditions in the intestine favors the formation of short chain fatty acids like propionic, butyric and acetic acids from intestinal microflora, which improves the metabolic activity in the intestine leading to increased absorption and availability of nutrients (Huda-Faujan *et al.,* 2010). This theory is supported by what was reported by Amaral *et al.,* (2009) who summarized the role of dietary protein in the maturation of the host immune system and the formation of antibodies.

Also, dietary protein has a very important role in organs responsible for immunity response (Jahanian, 2008). Moreover, Abdel Tawwab *et al.* (2010) reported

the importance of dietary protein in different physiological and immunological responses in Nile tilapia, which is adversely affected by any stress factor (s).

CONCLUSION

It can be concluded that lactic acid improved growth performance, including body weight, body length, weight gain, specific growth rate and survival; decreased feed conversion ratio, improved blood parameters and improved the nutritive value of whole fish as it increased crude protein and decreased fat content.

Also, using protected lactic acid showed more improvement in all tested parameters if compared with the value obtained from supplementation of non-protected lactic acid except for Hb, Htc, WBCs and RBCs, which was higher in the case of using non-protected acid, which is dissociated and absorbed, from the upper intestinal tract causing improvement of the vital activity of the body. Generally, lactic acid can be used as a growth promoter due to its acidifying activity either protected or non-protected and considering the protected one as the best promoting and protected one.

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ARABIC SUMMARY

تأثير حامض اللاكتيك المحمي وغير المحمي المستخدم كمحمض في عليقة أسماك البلطي النيلي

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أجريت الدراسة الحالية للمقارنة بين حامض اللاكتيك المحمي وغير المحمي على أداء النمو ومعدل الاستفادة من الغذاء وبعض المعايير الدموية والتحليل الكيماوي لأسماك البلطي النيلي. تم استخدام ثلاثة علائق متزنة الطاقة والمحتوى النيتروجيني بها (٣٧.٥٪ ± ٠.٩٧) بروتين خام (٤٤٢٧±٣٩ سعر حراري/جم) طاقة كلية. تم تدعيم العلائق بحامض اللاكتيك غير المحمي بنسبة ٠.٢٪ و٠.٢٪ من حامض اللاكتيك المحمي والعليقة المقارنة بدون إضافة بشكل فردي إلى ثلاث مجموعات سمكية متساوية (٢٥ سمكة) متوسط أوزان الجسم (٥.٤٢ ± ٠.٠٧ جرامًا) لمدة ٩٠ يومًا. أظهرت النتائج في نهاية التجربة أن المجموعة المقارنة معدلات نمو ومعدل الاستفادة من الغذاء أقل من حامض اللاكتيك المحمي وغير المحمي. بينما أظهرت الأسماك التي غذيت بحامض اللاكتيك المحمي أعلى وزن جسم نهائي، زيادة نهائية، متوسط الزيادة اليومية، معدل استهلاك العليقة ومعدل البقاء على قيد الحياة. وأظهرت الأسماك التي غذيت بحامض اللاكتيك المحمي تحسنا في خصائص الدم التي تم اختبارها مقارنة بالمجموعة الضابطة. تشير النتائج إلى أن العلائق المدعمة بحامض اللاكتيك في تغذية الأسماك يمكن أن تستخدم كمحمض لغرض تعزيز النمو. كذلك لحامض اللاكتيك تأثيرا كبيرا مقارنة مع اللاكتيك غير المحمي.