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Studies on Lysine Requirement for Growing mono-sex Nile Tilapia (*Oreochromisniloticus*)

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ABSTRACT

This study was conducted over 12 weeks' period in order to studythelysine requirements ofgrowing mono-sex Nile tilapia (*Oreochromisniloticus*). There were nine treatments (cont. T1 without lysine, T2=0.1 lysine, T3= 0.2lysine, T4=0.3 lysine, T5=0.4 lysine, T6=0.5 lysine, T7=0.6 lysine, T8=0.7 lysine, and T9=0.8 lysine). Each treatment consisting of three replicates. Initial body weight of fish was 10 g,stocking rate was10fish/aquariumand daily feeding rate was3% of fish live body weight. Fish were fed a balanced diet of 20% protein. The results indicated that the best fish production, chemical composition, and physiological status were obtained byT6 and T7 (level of the added lysine 0.5 and 0.6% of the diet, i.e. 1.74& 1.84 of the diet as total content of lysine**).**

INTRODUCTION

Tilapia is well adapted to enclosed water produces high yields and is one of the most important and extensively cultured fish species on the global scale. Among tilapia species, Nile tilapia (*Oreochromisniloticus*) is popular among farmers due to the advantages including fast–growing rate, short feeding cycles improved disease resistance, strong fertility and delicious taste.Owing to the increasing demand for tilapia in recent years, both quantity and good quality of food should be enhanced to meet the nutrition requirements of highly valued fish species for optimal aquaculture(Fitzsimmons, 2000).Tilapia are important culture fishes and knowledge of their amino acid requirements would be useful in formulating research and commercial diets, the amino acid requirements determined for the tilapia by Jaunce*yet al*. (1983) seem low in comparison with those of other warm water fishes, such as channel catfish and common carp (NRC,2011) and in comparison with the essential amino acid percentages in the muscle protein of the fish (Jaunce*yet al*.,1983).Protein is the costliest food ingredientin aquaculture. Estimating the amino acids of fish is important, because fish do not actually require dietary protein, but a rather well – balanced supply of dietary amino acids. The essential amino acid requirements have been established for only a few cultured fish species (NRC, 2011).Individual amino acid requirements for fish have been determined by using time – consuming and costly dose response feeding assays. As an alternative, the whole body composition of a species can be used to estimate simultaneously the requirements for all ten essential amino acids (Tacon, 1989).Lysine is an essential amino acid presents in high proportion in fish muscle tissue, involved in growth and maintenance of positive nitrogen balance, also used in"cross-linking" protein, especially collagen (UNM, 2006) and it is commonly the first – limiting essential amino acid (AA) in typical soybean meal. (Mavromichalis *etal*.,1998). Moreover, it plays an important role in the synthesis of carnitine, which is important for the transport of long – chain fatty acids into the mitochondrin for energy generation (Walton *etal*., 1984). Dietary lysine supplementation is related to advantages on weight gain, feel conversion, nitrogen retention and reduction in body lipid contents (Furuya*et al*., 2006). Therefore, the present work is performed to study the effect of dietary graded levels addition of lysine on growth performance, efficiency of feed utilization, carcass composition,and histological examination.

MATERIALS AND METHODS

Experimental fish, ingredients and diets:

The present work was conducted in Aquaculture Research Unit,Sakha, Kafr El-Sheikh during season 2014/2015 in order to evaluation thelysine requirement in Nile tilapia (*Oreochromisniloticus*) diet for good growth performance, feed utilization, body composition, amino acid retention, and histology examinationunder Egyptian conditions.

Fish culture system:

Fingerlings of mono-sex Nile tilapia (*Oreochromisniloticus*) were collected from a local fish farm Al Reyad, Tolompat 7, KafrEl – Sheikh prior to the start of the experiment.All collected fish were placed in fiberglass tank where they were fed a commercial diet for four weeks (acclimation period) under the laboratory conditions.The average initialbody weight of fingerlings was 10g/fish.The fish thereafter were divided into 9similar groups fed dietary protein level 20 %, fish were stocked at a rate of 10 fish in each aquarium, three repetitions represented each treatment The feeding trial was conducted in 27 glass aquaria. Each aquariummeasured 60 × 35 × 40 cm containing 70 lof water, supplied with well aerated and dechlorinated tap water and air stone. Light was controlled by a timer to provide 14h light: 10h dark as daily photoperiod. Fish feces and residual were removed by siphoning and about 50% of each aquarium water was daily replaced by well aerated freshwater.

Diet formulations:

A basal diet was formulated from the commercial ingredients (fish meal, soybean meal, yellow corn, wheat bran, vitamins and veg. oil and lysine.The dry ingredients were grounded then mixed through a feed

grinder to pellets of very small particle size (1 mm diameter).

Experiment diets: Nine diets were formulated based on different ingredients (Tables 2) and according to the recommendations for Nile tilapia (NRC2011). The ingredients were weighed and mixed by a dough mixer for 20 minutes. Graded levels of lysine wereadded (for all diets except the control diet)after homogenous mixture was obtained, forty ml water per hundred-gram diet was slowly added to the mixture according to Shimeino*et al*.(1993). The diets were cooked on water evaporator for 20 minutes. The diets were pelleted through fodder machine and the pellets were dried under room temperature for 24 h before used. The pellets were collected and saved in plastic bags and stored in refrigerator at 4 C° during the experimental period to avoid the deterioration of nutrients. The Fish were fed the experimental diets at a rate of 3 % of live bodyweight; the daily ration was introduced at 2 equal meals at 8 am and 2 pm through 12 weeks. The Fish were weighted at biweekly intervals during the experimented period and the feed quantities were readjusted according to the change in live body weight. The chemical analysis of feed ingredients used in the experiment tow diets is presented in Table 2. These diets were designated as diets 1 to 9, respectively and they were isonitrogenous (20 % CP), and isocaloric (4200 kal/Kg). Composition of the mixed diet is presented in Table 2. Lysine was added to this diet so as to reach the concentration level recommended by NRC 2011as the requirement for this species. The amino acids profile was undertaken by amino-acid analyzer in the central lab. of Faculty of Science, Cairo University (Table 3).

Table 1: The proximate analysis of the used ingredients (% on DM basis)

Ingred.	DM	CP	CF	Fiber	Ash	NFE*	GE** Kcal/diet	Lysine	Methionine
FM tuna	96.96	54.4	13.7	0.27	23.4	8.33	4698.16	4.62	1.95
BM	91.17	42.7	2.56	4.5	7.74	42.5	4396.7	2.94	0.46
WB	90.63	13.57	4.88	10.8	4.83	65.92	3934.41	0.66	0.15
YC	89.61	8.85	2.67	3.25	0.98	84.25	4213.89	0.23	0.08

***NFE= 100-(CP+EE+CF+Ash), ** Gross energy was calculated by multiplication the factor 4.1, 5.6 and 9.44 kcal GE/Kg DM, NFE, protein, fat, respectively (NRC 2011), FM=Fish meal, SBM=Soybean meal, WB=Wheat bran, YC=Yellow corn.**

Table 2: Ingredients and proximate analysis of the experimental diets used

Ingred.	T 1	T 2	T 3	T 4	T 5	T 6	T 7	T 8	T 9
FM[a]	10	10	10	10	10	10	10	10	10
SBM	18	18	18	18	18	18	18	18	18
WB	21	21	21	21	21	21	21	21	21
YC	45.64	45.54	45.44	45.34	45.24	45.14	45.04	44.94	44.93
Oil	3	3	3	3	3	3	3	3	3
Vitamin[b]	2	2	2	2	2	2	2	2	2
Lys.[c]	-	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Meth.[e]	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Sum.	100	100	100	100	100	100	100	100	100
CP	20	19.99	19.98	19.97	19.96	19.95	19.94	19.93	19.93
Lys.	1.24	1.34	1.44	1.54	1.64	1.74	1.84	1.94	2.4
Meth.	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
GE**	4280.65	4276.45	4272.25	4268.05	4263.85	4259.55	4255.35	4251.15	4250.75

a Tuna 60% crude protein powder, Country of Origin Yemen.

b Contained(asg/kgpremix): Thiamin-HCl,0.438;Riboflavin,0.632;Pyridoxine-HCl,0.908;D-pantothenicacid,hemicalciumsalt,1.724;Nicotinicacid,4.583;Biotin,0.211;Folicacid, 0.549; Vitamin B_{12}, 0.001; Inositol, 21.053; Menadione sodium bisulfite, 0.889; VitaminA acetate (500,000 IU/g), 0.677; Vitamin D3 (1,000,000 IU/g), 0.116; DL-alpha-tocopherylacetate (250 IU/g), 12.632; Alpha-cellulose, 955.58.

c L-Lysine 79 % HCl.

e DL-Methionine 99% (or) 2 Amino 4-Methionine Butauoie Acid CH11NO2S Eidesio, France.

**** Gross energy was calculated by multiplication the factor 4.1, 5.6 and 9.44 kcal GE/Kg DM.**

T1= without lys. Supplement, T2= T1+0.1 lys., T3=0.3lys., T4=0.4lys., T5=0.5lys., T6=0.6lys., T7=0.7lys., T8=0.8lys., and T9=T1+0.8 lys.

Histological examination:

Specimens from liver of the experimental fish were taken. Specimens were fixed immediately in 10 % neutral buffered formalin, then dehydrated in ascending grades of ethanol, embedded in paraffin, thenwere sectioned at 4 - 5 µmthickness and stained with haematoxylin and eosin (H, E) and examined by light microscope according to Bancroft and Gamble (2007)

Growth parameters:

Average total gain (ATG), average daily gain (ADG), specific growth rate(SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), proteinproductive value (PPV%) and survival rate (SR %) were calculated according to the following equation:

- ATG (g/fish) = {Average final weight (g) – Average initial weight (g)} as reported by (Annet.,1985).

- ADG(g/fish/day)={ATG(g)/experimental period (d)}.
- SGR (%/day) = {Ln final body weight – Ln initial body weight}× 100/experimental period (d) according to Pouomonge and Mbongland (1993).
- FCR = feed intake (g) / live weight gains as reported by De Selva and Anderson (1995).
- PER = live weight gain (g)/protein intake (g) as reported by De Selva and Anderson (1995).
- PPV (%) = 100{final fish body protein (g) – initial fish body protein (g) /crude protein intake (g).
- SR, % = 100{total No. of fish at the end of the experimental period/total No. of fish at the start of the experiment.

Table 3. Quantitative essential amino acid requirements of freshwater fish (DM basis) *Oreochro misniloticus* (%ofcrude protein).

Amino acid	*Oreochromis*ssp
Arginine	1.2
Histidine	1.0
Isoleucine	1.0
Leucine	1.9
Lysine	1.6
Methionine	0.7
Phenylalanine	1.1
Threonine	1.1
Tryptophan	0.3
Valine	1.5

NRC (2011) Nutritional Requirements of Fish and Shrimp

Proximate analysis:

Dry matter, crude protein, ether extract, (crude fiber) and ash contents of thetested ingredients and whole body of fish at the end of the experiment were performed according to A.OA.C. (1990).

Statistical analysis:

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F- test was significant, least significant difference was calculated according Duncan (1955).

RESULTS AND DISCUSSION

Growth Performance:

Averages BW of Nile tilapia during the experimental period as affected by the dietary L- lysine levels are presented in Table 4. Averages of initial weights (IW) at the start of the experiment had ranged between 11±0.00 and11±0.57g, with insignificant differences (P≥ 0.05) among the experimental groups.Averages of final weight (FW) for the experimental groups were significantly (P≤ 0.05) affected by dietary inclusion levels of L-lysine. The groups of fish fed diet 6 (diet1+0.5% Lys.) and diet 7 (diet1+0.6% Lys.) as well as diet 1 without lysine had significantly (P<0.05) higher final body weights.Table 4 revealed that the final body weight of Nile tilapia increased significantly (P<0.05) with each increment of lysine – HCl level up to diets 6 and over the recommended level of the 5thgroup (diet1+0.4 % Lys.). The differences in specific growth rate (SGR) which are presented in Table 4 at the end of experimental period revealed that the groups of fish fed diet 6 (dier1+0.5 %Lys.) and diet 7 (diet1+0.6 % Lys.) showed significantly (P<0.05) higher SGR than the other dietary groups, while group of fish fed diet 1 (without Lys.) had a significantly (P<0.05) lower SGR than the other dietary groups.The statistical analysis of results in Table 4 indicated that survival rate (SR) of Nile tilapia was notaffectedby the dietary lysine levels of addition.General results of Table 4 revealed that growth performance of Nile tilapia includingfinal weight (FW), average weight gain (AWG) and specific growth rate (SGR) wereimproved when diets contained 0.1 or 0.2% of lysine over the recommended lysine levels by NRC (2011).These results may lead to recommend thelysine requirements of Nile tilapia fingerlings as between 1.74 and 1.84% of the diet>These results are in accordance with those of Viola *etal* (1992) who reported that weight gain and SGR of Nile tilapia were the best when the diet contained 1.75% Lys.

Table 4. Growth performance of Nile tilapia fingerlings fed diets containing lysine addition for 12 week.

T	IW, g	FW, g	AWG, g	SGR, %/d	SR, %
1	11±0.57^{a}	27±1.21^{d}	16±1.16^{d}	1.42±0.65^{d}	100±0.00
2	11.±0.66^{a}	29.80±0.52^{c}	18.76±0.59^{c}	1.58±0.39^{c}	100±0.00
3	11±0.50^{a}	30.20±0.90^{c}	19.15±0.88^{c}	1.59±0.44a^{c}	100±0.00
4	11±0.00^{a}	31.43±0.58^{bc}	20.33±0.58^{bc}	1.65±0.29^{bc}	100±0.00
5	11±0.33^{a}	32.86± 0.49^{b}	21.83±0.49^{b}	1.74±0.24^{b}	100±0.00
6	11±0.57^{a}	35.60± 0.72^{a}	24.60±0.75^{a}	1.87±0.37^{a}	100±0.00
7	11±0.50^{a}	33.66±0.67^{ab}	22.61±0.62^{ab}	1.77±0.23^{ab}	100±0.00
8	11±0.66^{a}	31.46±0.43^{bc}	20.76±0.72^{bc}	1.68±0.34^{bc}	100±0.00
9	11±0.57^{a}	29.43±0.58^{c}	18.43±0.54^{c}	1.56±0.23^{c}	100±0.00

a-d: Means in the same column superscripted by different letters , significantly (P <0.05)

Viola andlahav (1994) reported that a diet containing 25% protein supplemented with 0.5% Lys.-HCL to contain 1.8 % total Lys., was quite enough for attaining the best growth performance results by carp and tilapia.Zaghloul (2005) reported that supplementing diets of growing all male mono-sex Nile tilapia with 10 to 20 % lysine over the requirements (1.59 and 1.71 %) improved significantly final weight, total weight gain, relative growth rate, daily weight gain and specific growth rate compared to the control group receiving 1.43 % lysine. Also, results revealed, the improvement in growth performance parameters was more pronounced in the group receiving 20% lysine over the requirements (1.71%).Akiyama *et al*.(1985)reported that based on growth data in Chum salmon fry, the requirements for threonine, histidine and lysine were

found out to be 1.2, 0.7 and 1.9 % of diet (dry weight basis), or 3.0, 1.6 and 4.8 % of dietary protein, respectively.Berge *et al*. (1998) reported that dietarylysine supplementation is related to advantages on weight gain.On the other hand, Furuya*et al*. (2012) reported that there was no effect of the dietary lysine levels on survival rate.However, the higher values of lysine requirement in experimental diets for Nile tilapia, maybe recuse several factors, such as the composition of the diets, feed management and statistical analysis used (Furuya*et al*., 2012).

Feed Utilization:

Averages of weight gain (AWG), feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) of Nile tilapia as affected with L-lysine-HCL supplemented levels in the diet are presented in Table 5.In general, these results revealed that average feed intake and weight gain increased with each dietary lysine level increase.These results are in agreement with the findings of Forster and Ogata (1998)who reported that lysine requirement of juvenile Japanese flounder (3.0 g initial weight) and red sea bream (1.7 g initial weight) was estimated by feeding six semi-moist diets (45–48% crude protein on dry basis) containing graded levels of lysine hydrogen chloride in replacement of sodium glutamate.El-Saidy and Gaber (2002) who reported that increasing the lysine level in tilapia diets from 1.63 to 2.05% increases the feed intake as results of improved weight gain. The same results were obtained byZaghloul (2005) who reported that increasing lysine level in Nile tilapia diets from 1.43 to 1.71% improved both FCR and PER. In this respect,El-Saidy andGaber (2002) reported also that highest FCR and PER records in Nile tilapia were obtain with a diet containing 2.05% Lys. level with 33% crude protein.

Table 5. Feed and nutrient utilization of Nile tilapia fingerlings fed diets containing graded levels of lysine for 12 Week.

Tre.	FI, g	FCR	PER
1	39.12±1.01^{d}	2.46±0.12^{a}	2.03±0.09^{d}
T2	41.08±0.74^{cd}	2.18±0.28^{b}	2.27±0.03^{c}
T3	42.12±1.50b^{cd}	2.19±0.23^{b}	2.26±0.02^{c}
T4	43.76±1.24a^{bc}	2.14±0.00^{b}	2.32±0.00^{bc}
T5	44.67±0.81^{abc}	2.04±0.00^{bc}	2.24±0.00^{ab}
T6	47.77±1.10^{a}	1.93±0.16^{c}	2.57±0.02^{a}
T7	46.10±1.25a^{b}	2.03±0.00^{bc}	2.45±0.00^{ab}
T8	44.40±1.27a^{bc}	2.13±0.01^{b}	2.34±0.02^{bc}
T9	40.84±2.08^{cd}	2.21±0.08^{b}	2.26±0.08^{c}

a-d: Means in the same column superscripted by different letters , significantly (P <0.05)

Body Composition:

The chemical composition of whole body of the experimental fish groups is presented in Table 6. The highest dry matter was observed in group of fish fed diet 1 (without lysine supplementation), the highest body crude protein was observed in group of fish fed diet 6 (diet1+0.5% Lyse. supplementation), the lowest body crude protein values were found in groups of fish fed diet 1 (without Lys supplemented), diet 2 (diet1+0.1 Lys.) and diet 3 (diet2+0.2 Lys.).From Table 6, average crude fat content was found significantly higher at the end of experimental for diets 1, 2, and 3.

Table 6. Mean values of body composition (% DM basis) of Nile tilapia fingerlings fed diets containgraded levels of lysine.

T	DM	CP	EE	FIBER	ASH	NFE
0	24.56±0.73a^{bc}	54.32±0.78^{d}	10.72±0.76^{b}	0.24±0.02^{bcd}	23.82±0.36^{b}	10.9±0.21^{b}
1	26.09±0.73^{a}	50.68±0.02^{e}	15.20±0.85^{a}	0.52±0.04^{ab}	21.59±0.36^{c}	12.01±0.21^{a}
2	25.89±0.49^{ab}	51.16±0.36^{e}	15.23±0.85^{a}	0.56±0.09^{a}	21.49±0.36^{c}	11.56±0.21^{a}
3	25.48±0.41^{ab}	51.24±0.44^{e}	15.41±0.87^{a}	0.31±0.04ab^{cd}	20.25±0.36^{d}	12.79±0.41^{a}
4	25.12±0.57^{abc}	55.15±0.77^{cd}	12.17±0.75^{b}	0.18±0.00^{cd}	21.79±0.36^{c}	10.71±0.21^{b}
5	24.55±0.54^{abc}	57.31±0.87^{ab}	11.74±0.61^{b}	0.13±0.01^{cd}	21.67±0.36^{c}	9.15±0.24^{c}
6	24.79±0.82a^{bc}	58.22±0.54^{a}	11.57±0.61^{b}	0.05±0.01d	22.44±0.36^{c}	7.72±0.21^{d}
7	23.88±0.27^{bc}	54.17±0.71^{d}	8.44±0.75^{d}	0.14±0.00^{cd}	27.28±0.36^{a}	9.97±0.21^{c}
8	25.29±1.00^{ab}	56.23±0.58^{bc}	16.38±0.74^{a}	0.15±0.00^{cd}	20.40±0.36^{d}	6.84±0.21^{e}
9	23.15±0.34^{c}	53.63±0.73^{d}	11.87±0.45^{b}	0.38±0.25a^{bc}	22.55±0.36^{c}	11.57±0.21^{a}

a- d: Means in the same column superscripted by different letters , significantly (P <0.05)

Histological findings:

Histological evaluation of the liver of fish supplemented with lysine at concentration (level 1 and 2 of **NRC,** respectively) showed marked hepatocytes vacuolation (Figs. 1& 2). The vacuolation of hepatocytes tended to decrease especially in diet supplemented with lysine at concentrationlevels 3, 4 and 5 of NRC,respectively (Figs. 3, 4, and 5). The level 6 of supplemented diet with lysine at showed mild vacuolation of hepatocytes. The vacuolation was obviously pushing the nuclei of the hepatocytes toward the blood sinusoids (Fig. 6). Interestingly, increase of the lysine supplementation in the diet starting from the level 7 showed increase in the hepatocytes vacuolation

in dose-dependent manner. There was also loss of zymogen granules of the pancreatic portion with degeneration of pancreatic cells with deposition of heamosiderin brown pigments within the pancreatic portion (Fig. 7). The 8th and 9th levels of lysine revealed marked hepatocytes vacuolation with marked degeneration pancreatic portion (Figs. 8& 9).

Regarding to kidney, similarly, the 1st and 2nd levels of lysine showed vacuolation of renal tubular epithelium (Figs. 10& 11). Increase of lysine supplementation as in levels 3, 4, 5, 6 and 7 demonstrating decrease in vacuolation of renal tubular epithelium (Figs. 12, 13, 14, 15, and 16). The other levels of 8 and 9 showed mild vacuolation and was within the normal limits (Fig. 17, 18).

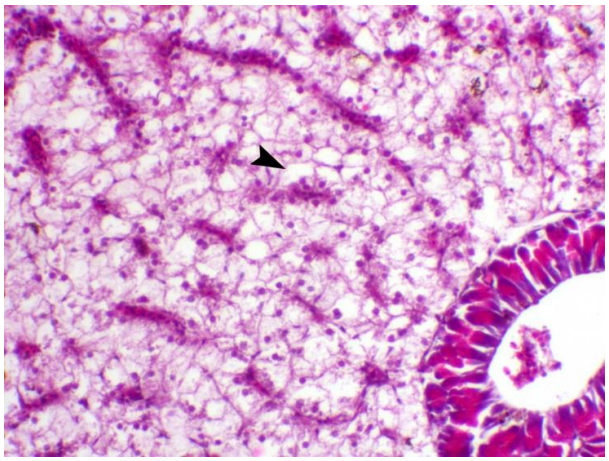

Fig. 1. Liver of fish supplemented with lysine at level 1 of NRC showed marked hepatocytes vacuolation, H&E, X200.

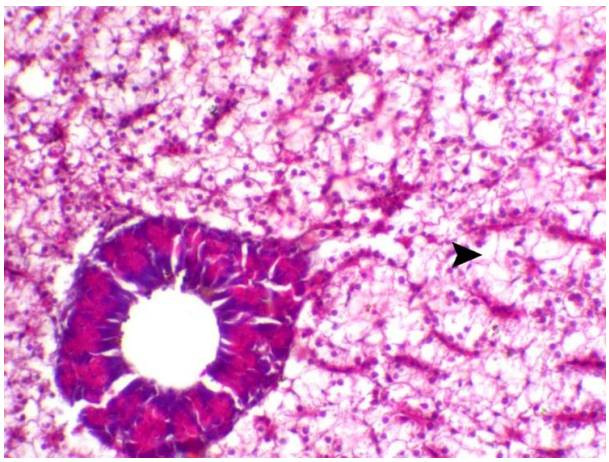

Fig. 2. Liver of fish supplemented with lysine at level 2 of NRC showed marked hepatocytes vacuolation, H&E, X200.

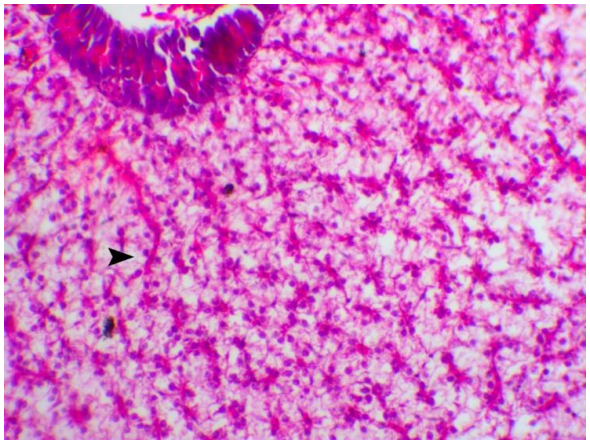

Fig. 3. Liver of fish supplemented with lysine at level 3 of NRC showed mild hepatocytes vacuolation, H&EX200.

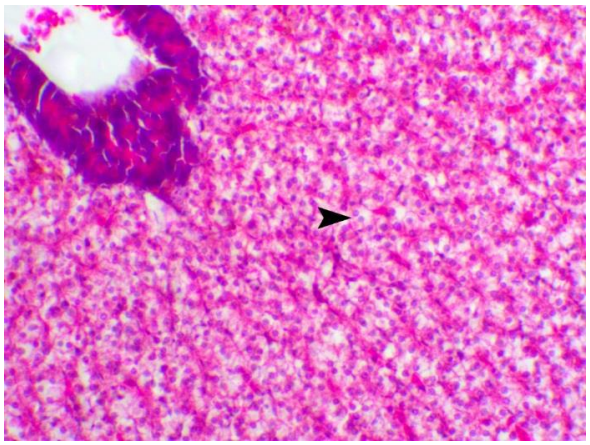

Fig. 4. Liver of fish supplemented with lysine at level 4 of NRC showed mild hepatocytes vacuolation, H&E, X200.

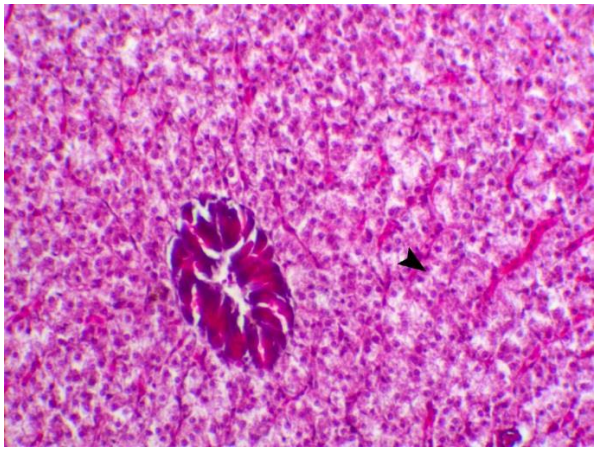

Fig. 5. Liver of fish supplemented with lysine at level 5 of NRC showed mild hepatocytes vacuolation, H&E, X200.

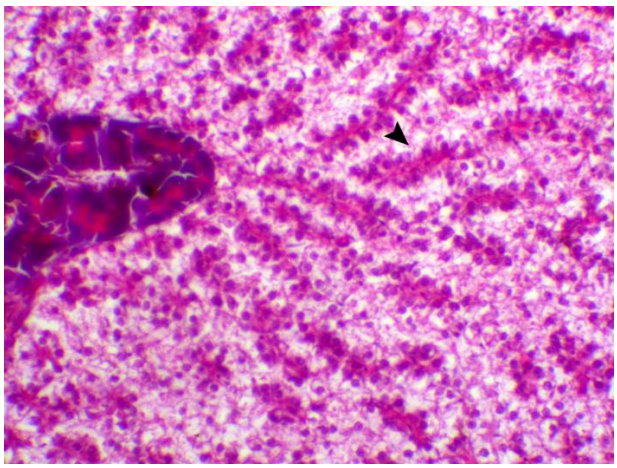

Fig. 6. Liver of fish supplemented with lysine at level 6 of NRC showed increase of hepatocytes vacuolation, H&E, X200.

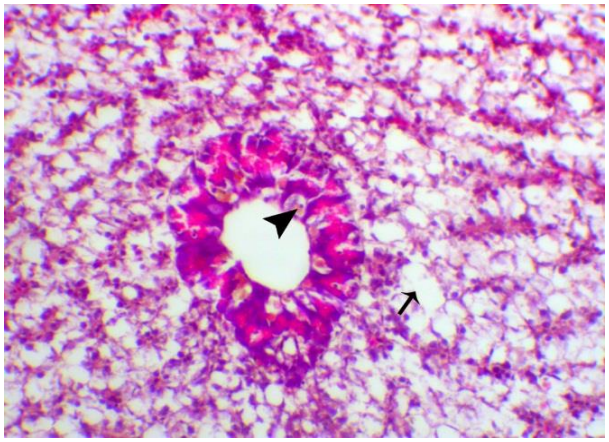

Fig. 7. Liver of fish supplemented with lysine at level 7 of NRC showed marked increase of hepatocytes vacuolation (arrow) with deposition of haemosidrin pigment within the pancreatic portion (arrowhead), H&E, X200.

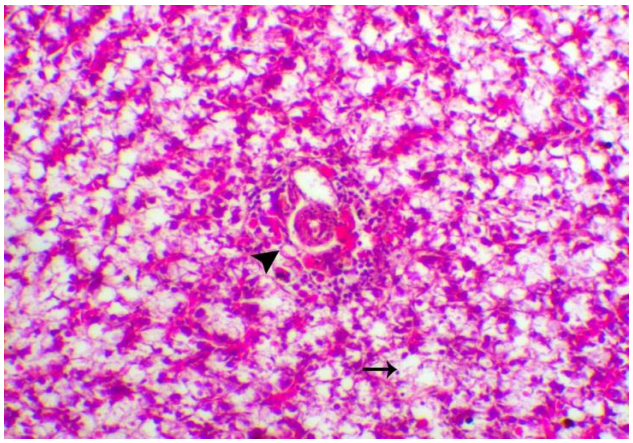

Fig. 8. Liver of fish supplemented with lysine at level 8 of NRC showed marked increase of hepatocytes vacuolation (arrow) with degeneration of pancreatic portion (arrowhead), H&E, X200.

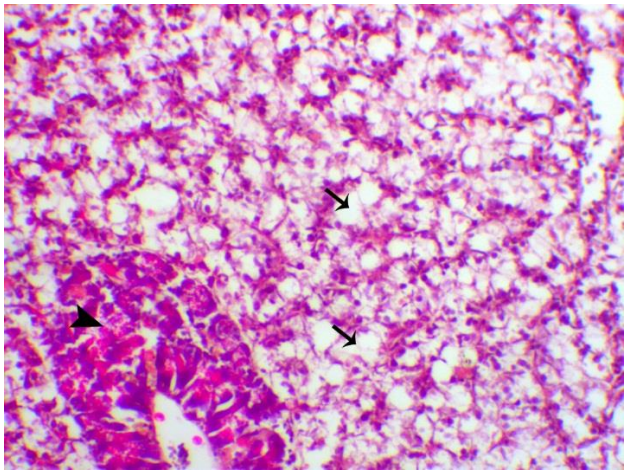

Fig. 9.Liver of fish supplemented with lysine at level 9 of NRC showed marked increase of hepatocytes vacuolation (arrow) with degeneration of pancreatic portion (arrowhead), H&EX200.

CONCLUSION

It was proved that Nile tilapia fed diet containing lysine addition at 0.5 and 0.6 % of the diet gave the best results at the end of the experiment, concerning fish production and quality.

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دراسات عن احتياجات أسماك البلطي النيلي وحيد الجنس النامية من الليسين.

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اجريت هذه الدراسة لمدة ١٢أسبوعا وذلك لدراسة الاحتياجات الغذائية لأسماك البلطى النيلى وحيد الجنسمن الحامض الأمينى الليسين. وقد استخدمت أحواض زجاجية ووُزعت بها الأسماك بعدد ١٠ سمكات للحوض، بوزن ابتدائى ١٠ جرام للسمكة، وغُذيت الأسماك يوميا لمدة ٦ أيام أسبوعيا بمعدل ٣% من الوزن الحى للأسماك، واستُخدمت ٩ تركيزات (% من العليقة) للإضافات من الليسين وكانت كالآتى: ١= كنترول عليقة خالية من إضافة الليسين،٢= ١.٠ ٣= ٢و. ٤= ٣و. ٥= ٤و. ٦=٥و. ٧=٦و. ٨=٧و. ٩=٨و. وكانت نسبة البروتين فى العلائق ٢٠%. وكانت أهم النتائج هىالحصول على أفضل معدلات نمو وتحليل كيماوى وقياسات دمويةفى المجاميع التى تغذت على علائقتحتوى على ليسين مضاف بنسبة٠.٥ و ٠.٦.