EFFECT OF DIETARY HIGH LYSINE AND METHIONINE LEVELS ON GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) FED ON DIET AT DIFFERENT FREQUENCIES.

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(Received 26/7/2005, accepted 20/10/2005)

SUMMARY

The present study was curried out at the aquaculture experimental station, Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt during the period from 1/7 to 22/9/2004. A total number of 480 Nile tilapia fingerlings weighing 10.4 ± 0.4g/ fish were distributed into six experimental groups representing two lysine + methionine levels (the requirement and 30% over the recommended level). Within each amino acid level three feeding frequencies (2, 4 and 6 times daily) were tested. The experiment was conducted in fiber glass tanks, each of 1 m^3 total water volume supplied with aeration source in a recirculating closed water system. The applied treatments were performed in replicate tanks each with 40 fish. The experiment lasted 12 weeks after start. Results obtained are summarized in the following:

- Supplementing growing Nile tilapia with both lysine and methionine to reach 30% over the requirements improved significantly final weight, weight gains, protein productive value and energy utilization, however it had insignificant effects on feed conversion ratio and specific growth rate.
- Increasing feeding frequency from 2 to 4 or 6 times daily improved significantly final weight; weight gains and released significant effect on feed conversion ratio, specific growth rate and nutrient utilization parameters.
- Both amino acids supplementation and feeding frequency released significant effects on chemical composition of whole tilapia bodies.

Keywords: *Nile tilapia, lysine supplementation and feeding frequency.*

INTRODUCTION

It has been reported that addition of supplemental methionine and lysine improved growth in common carp (*Cyprinus carpio*) fed diets with soy flour (Murai *et al.* 1982, 1989). Nordrum *et al.* (2000) reported that higher protein utilization in fish may results from increasing protein digestibility, improving amino acid balance and reducing the amount of protein used for energy production. Plant protein sources could be used to provide a significant proportion of protein requirements of Nile tilapia (El-Duhhar and El-Shazly,

1993). However, one of the factors limiting their use at higher levels appeared to be the deficiencies of certain essential amino acids, particularly methionine and lysine for tilapia mossambicus (Jackson and Copper, 1982). Viola *et al.* (1992) conducted an experiment where protein contributed by soybean meal in 30% protein for carp and tilapia feeds. They formulated five feeds of 3900 Kcal gross energy /kg which were compared each with (A) 30% protein/ 1.8 total lysine; (B) 25% protein + 0.5% lysine-HCI with 1.8% total lysine (C) 25% protein/ 1.4% total lysine; (D) 20% protein + 1.0% lysine-HCL/ 1.8 total lysine (E) 20% protein + 0.5 lysine-HCL with 1.4 total lysine. They reported that group B attained weight gains equal to group A, whereas groups C, D and E lagged 20% or more. The same authors concluded that 5% of the soybean meal in a 30% protein carp feed could be replaced by 0.5 lysine-HCL and 4.5 grains without impaired growth and resulting in 20% less nitrogen excretion. They reported also that further reduction of feed protein to 20% impaired weight gains, in spite proper lysine supplementation. The same authors tested four feeds with four replicates on tilapia (A) 30% protein with 1.75% lysine (B) 30% protein + 0.5% lysine HCL and 2.1% total lysine (C) 25% protein 1.4% total lysine (D) 25% protein + 0.5% lysine HCL with 1.75% total lysine. They found that the weight gains of both 30% protein groups (A and B) surpassed those with 25% protein groups (C and D). They concluded that supplemental lysine did not yield any growth response at both protein levels and thus failed to reduce nitrogen excretion. Deyab and El-Said (2002), replaced fish meal by soybean meal supplemented by L-lysine at 0.5; 1.0; 1.5 and 2%. They found that the best growth performances were recorded with diet containing 55% SBM and 0.5% lysine. The authors suggested that a diet contained 55% SBM; supplemented with 0.5% lysine; can be totally replace fish meal in a diet for Nile tilapia fingerlings without adverse effects on its performance.

The rate of feed consumption is a function of environmental condition, species, dietary composition, meal size, fish size and feeding frequency (Dos Santos and Jobling, 1995; Wang *et al.* 1998; Liu and Liao, 1999 and Riche, 2000). Feeding intervals or feeding frequency is strongly correlated with gastric evacuation time (Holmgren *et al.* 1983 and Lee *et al.* 2000), and the return of appetite is closely related to the rate of gastric emptying.

Gastric evacuation rate is also a function of temperature, fish weight, meal size, dietary composition and energy and feeding frequency (Windell *et al.* 1969; Grove *et al.* 1978; Flowerdew and Grove, 1979; Grove and Crawford, 1980; Jobling, 1980; Persson, 1981; Hofer *et al.* 1982 and Holmgren *et al.* 1983). Riche *et al.* (2004) evaluated the effects of feeding frequency (3 or 5 meals/day) on gastric evacuation and the return of appetite in Nile tilapia. They reported that fish fed at 4-5-h intervals consumed as much as they have evacuated and fish receiving meals at 2-3-h intervals exhibit gastric over load.

The purpose of the present investigation is to study the effect of lysine and methionine supplementation as well as feeding frequency on growth and feed utilization traits of growing Nile tilapia (*Oreochromis niloticus*) under tank culture conditions.

MATERIALS AND METHODS

This study was carried out at the intensive fish production experimental unit belonging to Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. The

study aimed to investigate the effect of increasing both lysine and methionine dietary levels by 30% over the NRC (1993) recommended levels on growth and feed utilization parameters of growing Nile tilapia (*Oreochromis niloticus*). Also the effects of feeding frequencies (2, 4 and 6 times) on growth parameters daily were studied.

Experimental fish:

A total number of 480 monosex Nile tilapia fish purchased from a private Nile tilapia hatchery in Kafr El-Sheikh governorate were used in this study. Average initial weight of the experimental fish was 10.4g ± 0.4 at the experimental start. The fish were acclimated for one week to the experimental tank conditions before they were distributed into the experimental groups. During the acclimation period prior to the experiment all fish were fed on the control diet for a week.

Experimental diets:

An experimental Nile tilapia diet (Table 1) was formulated to contain 34.3% crude protein and 4495.30 Kcal/Kg gross energy. The calculated lysine and methionine from the ingredients used in unsupplemental diet were 1.61 and 0.52%, respectively. Crystalline amino acids were supplemented at 0.32% lysine and 0.46% methionine which presenting 30% over the recommended levels (1.43 and 0.75%) for both amino acids according to NRC (1993). The experimental diets were fed at a daily rate of 3% of fish biomass in each tank. The supplemental L-lysine and Dl-methionine were incorporated in the diet by replacing part of the wheat bran component. The daily diet was fed at frequencies of 2, 4 and 6 times daily to test the effect of feed frequency. The diets were manufactured in form of pellets (3mm) using a mincing machine with 3mm die. Twelve experimental fiber glass tanks, each 2m length; 1m width and ½m depth were used in this study. The twelve tanks represented two diets (un-supplemented with lysine and methionine) and three feeding frequencies (2, 4 or 6 times daily) in two replicates. The tanks were filled with dechlorinated tap water in a complete water recycling system. Tanks were provided with air via an air compressor for 24 hours. The experiment started at 1/7/2004 and lasted 12 weeks after start. The experimental tanks were exposed to natural day light during the period of experiment i.e 14 hr. light and 10 hr. darkness.

Diet and fish body analysis:

At the beginning of the feeding trail 20 fish were sampled and stored at – 20 °C for the analysis of whole body composition as a zero group. At the end of the experiment period, samples of fish per tank were with drawn for chemical analyses. The chemical composition of the experimental diet and whole fish bodies including moisture; crude protein (CP); ether extract (EE) and ash was carried out according to the methods described by the AOAC (1995). Nitrogen free extract (NFE) contents were calculated by differences. Gross energy (GE) contents of the diets and the fish bodies were calculated according to Jobling (1983) using the multiplication factors of 4.0; 5.65 and 9.45 kcal GE/g for carbohydrate; protein and fat, respectively.

Sampling and data collection:

All fish in each treatment with its replicate were weighed individually at the experimental start and every two weeks thereafter. The amount of feed was adjusted according to the new fish weight every two weeks.

Average weight gain (AWG), average daily gain (ADG), specific growth rat (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and energy

Table (1) : Formulation and proximate analysis (on DM %) of the experimental diet fed to monosex Nile tilapia (*Oreochromis niloticus*).

Ingredients	Component %
Yellow corn	48.25
Soybean meal (44%)	23.25
Fish meal (72%)	14.00
Wheat bran	8.72
Sunflower oil	2.00
Vit. & Min. mixture [1]	3.00
L- lysine HCl (78.8%)	0.32
DL- methionine (99%)	0.46
Total	100
Proximate analysis (%)	
DM	84.80
CP	34.30
EE	7.90
CF	5.50
Ash	7.03
NFE [2]	45.27
Gross Energy (Kcal/ Kg) [3]	4495.30

(1) Each 1 Kg contains Vit. A 4.8 mIU; E 4g; K 0.8 g; B_1 0.4 g; B_2 1.6g; $B_6$0.6 g; B_{12} 4g; Pantothenic acid 4g; Nicotinic acid 8 g; Folic acid 400 mg; Biotin 20 mg; choline chloride 299 g; copper 4g; Iodine 0.4g; Iron 12g; Manganese 22g; Zinc 22g and selenium 0.04g.

(2) NFE = 100 – (Crude protein + Ether extract + crude fiber + crude ash).

Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, 4.0 and (Cal GE/g DM) for crude protein, ether extract and nitrogen free extract, respectively (Jobling, 1983).

utilization (EU) were calculated according to the following equations:

1. AWG (g/fish) = [Average final weight (g) – Average initial weight (g)]
2. ADG (g/fish/day) = [AWG (g)/ experimental period (d)]
3. SGR (%/day) = [(Ln final weight (g) – Ln initial weight (g)) × 100]/experimental period (d).
4. FCR = Feed intake, dry weight (g) / live weight gain (g)
5. PER = Live weight gain (g) / protein intake (g)
6. PPV(%) = 100 × [Final fish body protein (g) – initial fish body protein (g)/ crude protein intake (g)].
7. EU(%)= (Retained energy Kcal/energy intake, Kcal) × 100

Statistical analysis of data:

The data were statistically analyzed by using SAS programme, (1999) SAS/STAT user's guide SAS inst. Inc. Cary NC. USA. Differences between treatments were conducted according to Duncan, (1955). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance parameters:

Results of Table (2) show that averages of initial body weights had ranged between 10.08 and 10.82g with insignificant differences among the experimental groups. As presented in Table (2) averages of final weight at termination of the experiment (12 weeks after start) were found to be 79.72; 84.70; 89.96; 82.34; 87.72 and 91.79 g for T_1, T_2, T_3, T_4, T_5 and T_6, respectively. Analysis of variance for final weight indicated clearly that final weights improved significantly ($P<0.05$) with lysine and methionine supplementation and with increasing feeding frequency with the un-supplemented and supplemented groups (Table 2). Regardless of feeding frequency (Table 3), averages of final weights as affected by lysine and methionine supplementation were found to be 84.79 and 87.28g for the un-supplemented and supplemented groups, respectively with significant differences ($P<0.05$) for the favor of amino acids supplementation. Irrespective of amino acids supplementation (Table 4), averages final weight affected by feeding frequencies 2, 4 or 6 times/day were 81.03; 86.21 and 90.88 g, respectively and analysis of variance of the results indicated that averages final weight improved significantly ($P<0.05$) with increasing the daily feeding frequency. The same trends were observed by the average weight gain (AWG) and the averages of daily gains (ADG) where amino acids (lysine + methionine) supplementation and increasing feeding frequencies from 2 to 4 or 6 times/daily resulted significant ($P<0.05$) improvements in both AWG and ADG (Tables 2 and 3). The results are in agree with those reported by Gomaa (1995) and El-Saidy and Gaber (2002), who reported that supplementation of methionine and lysine to Nile tilapia diets containing high percentages of soybean meal resulted in the highest growth performance parameters. In this connection, Santiago and Lovell (1988) recommended for *O. niloticus* a lysine level of 5.12% of the dietary protein (1.43% of the diet) for maximum growth performance and utilization of feed. Santiago (1986) reported that young tilapia fish required 5.61% lysine as percentage of protein for maximum growth which correspond 1.57% of the whole diet. Results of Tables (2 and 3) are also in accordance with the findings of Shiau *et al.* (1987), who reported that addition of supplemental methionine improved growth of tilapia. Also Murai *et al.* (1986) reported that the nutritional value of soy flour was improved by

Table (2) : Growth Performance and nutrients utilization of monosex Nile tilapia (*Oreochromis niloticus*) fed the experimental diets.

Item	Diets					
	Without supplemental amino acids			With supplemental amino acids		
	Number of feeding/ day					
	2	4	6	2	4	6
	T_1	T_2	T_3	T_4	T_5	T_6
Average initial weight (g)	10.08 ±0.03	10.82 ±0.03	10.08 ±0.01	10.43 ±0.02	10.54 ±0.01	10.76 ±0.02
Average final weight (g)	79.72^{f} ±0.36	84.70^{d} ±0.42	89.96^{b} ±0.42	82.34^{e} ±0.48	87.72^{c} ±0.47	91.79^{a} ±0.39
AWG (g)	69.64^{f} ±0.01	73.88^{d} ±0.42	79.88^{b} ±0.42	71.91_{e} ±0.47	77.18^{c} ±0.47	81.03^{a} ±0.39
ADG (g)	0.83^{f} ±0.01	0.88^{d} ±0.01	0.95^{b} ±0.01	0.86^{e} ±0.01	0.92^{c} ±0.01	0.97^{a} ±0.01
SGR (%/day)	2.46^{d} ±0.01	2.45^{d} ±0.01	2.61^{a} ±0.06	2.46^{d} ±0.01	2.52^{c} ±0.01	2.55^{b} ±0.01
Feed Consumption (g)	84.50^{f} ±0.09	89.50^{d} ±0.05	95.45^{b} ±0.08	87.25^{e} ±0.12	92.25^{c} ±0.05	106.10^{a} ±0.15
FCR	1.21^{a} ±0.01	1.21^{a} ±0.01	1.20^{a} ±0.01	1.21^{a} ±0.01	1.20^{a} ±0.01	1.31^{b} ±0.01
PER	2.40^{b} ±0.01	2.41^{ab} ±0.01	2.44^{a} ±0.01	2.40^{b} ±0.01	2.43^{ab} ±0.01	2.23^{c} ±0.01
PPV (%)	30.99^{e} ±0.16	32.57^{d} ±0.18	34.15^{b} ±0.20	33.31^{c} ±0.20	34.88^{a} ±0.22	33.28^{c} ±0.16
EU (%)	24.14^{e} ±0.12	25.37^{d} ±0.14	26.71^{b} ±0.14	26.14^{c} ±0.15	27.32^{a} ±0.16	26.05^{c} ±0.13

a, b, c etc: Means in the same row with different superscripts are significantly different ($P< 0.05$).

Table (3) : Effects of lysine + methionine supplementation on growth performance and nutrients utilization of monosex Nile tilapia (*Oreochromis niloticus*) fed the experimental diets.

Item	Effect of supplemental amino acids	
	Without supplementation	With supplementation
Average initial weight (g)	10.33 ±0.05	10.58 ±0.02
Average final weight (g)	84.79[b] ±0.59	87.28[a] ±0.56
AWG (g)	74.47[b] ±0.59	76.71[a] ±0.55
ADG (g)	0.89[b] ±0.01	0.92[a] ±0.01
SGR (%/day)	2.51 ±0.01	2.51 ±0.01
Feed consumption (g)	89.82[b] ±0.58	95.27[a] ±1.04
FCR	1.21 ±0.00	1.24 ±0.01
PER	2.42[a] ±0.01	2.35[b] ±0.01
PPV (%)	32.57[b] ±0.20	33.82[a] ±0.15
EU (%)	25.41[b] ±0.16	26.50[a] ±0.11

a, b, c etc: Means in the same row with different superscripts are significantly different (P< 0.05).

addition of 0.4% crystalline L. methionine. On the other hand, Andrews and Page (1974) reported that methionine supplementation to channel catfish diets resulted in no improvement in weight gains which may due to the higher levels of sulpher amino acids in the basal diet fed.

Concerning feeding frequency (Tables 2 and 4), results of the present study revealed that final weights, average weight gain (AWG) and ADG of Nile tilapia were improved with each increase in feeding frequencies which are in accordance with results reported by Wang *et al.* (1998). They reported that cumulative feed consumptions, final weight, weight gain and SGR of hybrid sunfish were significantly greater in fish fed three and four times daily compared to those fed once daily. Also studies conducted on other fish species have also showed that feed consumption and fish growth generally increased with increasing feeding frequency up to a given limit (Andrew and Page 1975 on catfish; Grayton and Beamish 1977 on rainbow trout; Siraj *et al.* 1988 with red tilapia and Tsevis *et al.* 1992 on sea bass.

As presented in Table (2), averages of specific growth rates (SGR) were significantly ($P<0.05$) higher in T_3; T_5 and T_6 compared to T_1; T_2 and T_4. Furthermore, T_3 showed the highest SGR ($P<0.05$) followed in a significant decreasing order ($P<0.05$) by T_6 and T_5. Regardless of feeding frequency (Table 3), amino acids (lysine and methionine) supplementation seemed to release no significant effects on SGR of Nile tilapia. On the other hand, SGR recorded significantly improvement ($P<0.05$) with increasing the times (frequency) of feeding, regardless of amino acids supplementation (Table 4). These results are in accordance with the findings of Siraj *et al.* (1988) and Wang *et al.* (1998), who reported that increasing feed frequency improved growth parameters including SGR.

Feed utilization parameters:

Results of Table (2) showed that FC increased ($P<0.05$) with lysine and methionine supplementation and with increasing the feeding frequency. As presented in Table (3) FC improved significantly ($P<0.05$) with lysine + methionine supplementation, regardless of feeding frequency. Moreover, FC averages (Table 4) were found to be 85.88; 90.98. and 100.77g for feed frequencies 2, 4 and 6 times daily, respectively, regardless of amino acids levels. Results concerning feed intake as affected by amino acids (lysine + methionine) obtained in the present study are in accordance with those reported by El-Saidy and Gaber (2002), who showed that increasing lysine level in Nile tilapia diets from 1.63 to 2.05% increased feed intake as a result of improved weight gain. Also results of Tables (2 and 3) are in accordance with Viola *et al.* (1982) and Shiau *et al.* (1989), who reported that methionine supplementation in carp and hybrid tilapia improved feed consumption. Results concerning feeding frequency and its positive significant effect ($P<0.05$) on FC (Tables 2 and 4) are in complete agreement with the findings of Andrews and Page (1975); Grayton and Beamish (1977); Siraj *et al.* (1988). Tsevis *et al.* (1992) and Wang *et al.* (1998); who reported that increasing feed frequency in some fish species up to a given limit generally increased feed consumption and fish growth.

Average of feed conversion ratio FCR (Table 2) showed that T_1; T_2; T_3; T_4 and T_5 recorded significantly ($p< 0.05$) better (lower) FCR values compared to T_6. These results may indicate that group fed on the lysine + methionine supplemented diet and fed the diet at the highest frequency showed the highest (worth) FCR values. On the other hand, as

Table (4) : Effects of feeding frequency on growth performance and nutrients utilization of monosex Nile tilapia (*Oreochromis niloticus*) fed the experimental diets.

Item	Effect of feeding frequency		
	2	4	6
Average initial weight (g)	10.26 ±0.03	10.68 ±0.03	10.42 ±0.05
Average final weight (g)	81.03^{c} ±0.36	86.21^{b} ±0.39	90.88^{a} ±0.32
AWG (g)	70.78^{c} ±0.34	75.53^{b} ±0.41	80.46^{a} ±0.30
ADG (g)	0.85^{c} ±0.00	0.90^{b} ±0.00	0.96^{a} ±0.00
SGR (%/day)	2.46^{c} ±0.00	2.49^{b} ±0.01	2.58^{a} ±0.01
Feed consumption (g)	85.88^{c} ±0.23	90.98^{b} ±0.24	100.77^{a} ±0.85
FCR	1.21^{a} ±0.00	1.21^{a} ±0.01	1.26^{b} ±0.01
PER	2.40^{a} ±0.01	2.42^{a} ±0.01	2.34^{b} ±0.02
PPV (%)	32.15^{b} ±0.22	33.73^{a} ±0.29	33.72^{a} ±0.15
EU (%)	25.14^{b} ±0.18	26.35^{a} ±0.19	26.38^{a} ±0.11

a, b, c etc: Means in the same row with different superscripts are significantly different (P< 0.05).

illustrated in Table (3), lysine + methionine supplementation released insignificant effects on FCR of Nile tilapia. Moreover, FCR values for groups fed the diet 6 times daily were significantly ($p< 0.05$) higher (worth) than those fed 2 or 4 times daily, regardless of amino acids supplementation (Table 4). These results may be explained by the findings of Wang *et al.* (1998), who reported that both feed consumption and growth rates appeared to increase with the number of meals per day up to three meals; further increases in feeding frequency did not resulted in greater growth and consequently in bad FCR records.

In this connection, it is well known that social interactions and dominance hierarch formation can lead to the suppression of feed intake and growth of subordinate individuals (McCarthy *et al.* 1992 and Jobling, 1994), which may be the case in the group fed 6 meals a day in this study, thus 6 meals/ day may increased the competition on the meal because the low amount of diet offered each time in the 6 meal group.

Results of PER (Table 2) show that the best PER values were obtained by T_3 (fed the unsupplemented diet 6 times/ day) followed in a significant decreasing order ($p< 0.05$) by T_1, T_4 and T_6, however differences in PER among T_3; T_2 and T_5 were insignificant. Regardless of feeding frequency (Table 3), averages of PER as affected by amino acids (lysine + methionine) supplementation were found to be 2.42 and 2.35 for the unsupplemented and supplemented groups, respectively. Difference in PER were significant for the favor of the unsupplemented group. These results may due to the significant interaction between amino acid supplementation and feed frequency. Furthermore, both feeding frequencies 2 and 4 times daily had significantly ($p< 0.05$) higher PER values compared to 6 times daily, regardless of amino acids supplementation (Table 4). These results are in accordance with the findings of Viola *et al.* (1992), who reported that increasing lysine level from 1.75 to 2.1% in Nile tilapia diet released negative effects on protein retention as well as protein utilization parameters (PER and PPV). Also Gomaa (1995) reported that supplementing Nile tilapia diet containing soybean meal in replacement with fish meal (100; 50; 25; 0% soybean meal) with methionine and lysine did not release significant effects on PER and FCR. On the other hand, El-Saidy and Gaber (2002) reported that a dietary lysine level 2.05% in diets of Nile tilapia containing 33% CP improved significantly both FCR and PER values. In agreement with results of the present study Shiau *et al.* (1989) found that supplementing methionine to *O. niloticus* × *O. aureus* diet containing 67% fish meal and 33% soybean meal or 33% fish meal and 67% soybean meal did not significantly improve FCR, PER and protein digestibility. Also Viola *et al.* (1988) noticed that adding L-methionine and L-lysine HCl to diets of hybrid tilapia containing 25% fish meal and 75% soybean meal protein did not improve FCR and PER. Results concerning the effect of feeding frequency of the present study are also in partial agreement with the finding of Lee *et al.* (2000), who reported that different feeding frequencies had no significant effects on PER of Korean rockfish.

Results of PPV% (Table 2) showed that the highest ($p< 0.05$) value was attained by T_5 followed in a significant decreasing order ($P<0.05$) by T_3; T_4 and T_6; T_2 and T_1, respectively. These results indicate that tilapia group fed the amino acids (lysine and methionine) supplemented diets at 4 times/ day was able to utilize the dietary protein more

efficient than the other treatment groups. As presented in Table (3) fish fed the lysine + methionine supplemented groups showed higher ($p< 0.05$) PPV% values than those fed the unsupplemented diets. On the other hand, Nile tilapia fed 4 or 6 times daily showed significantly ($P<0.05$) better PPV% values (Table 4). These results are in partial agreement with the finding of Webster *et al.* (1992b) with channel catfish. Results of energy utilization efficiency % (Table 2), revealed that the highest EU% was obtained by fish group T_5; followed in a significant ($P<0.05$) decreasing order by T_3; T_4 and T_6; T_2 and T_1, respectively. As presented in Table (3) EU% of tilapia fed the lysine + methionine supplemented diet had significantly ($p< 0.05$) higher EU% than those fed on the unsupplemented diet. On the other hand, results of Table (4) indicated that both fish fed the diet 4 or 6 times daily had significantly ($P<0.05$) higher EU% compared to those fed 2 times/ day.

Body chemical composition:

As presented in Table (5) averages of dry matter DM%; crude protein (CP); either extract (EE), ash and energy contents Kcal/kg of fish bodies calculated on dry matter basis at the experimental start were found to be 19.73; 51.42; 24.32; 23.21% and 5204.0 Kcal/ kg, respectively. At experimental termination T_6 showed the highest ($P<0.05$) DM contents followed in a significant decreasing order by T_5; T_3; T_4; T_2 and T_1, respectively. Regardless of feeding frequency, results of Table (5) show that supplementing growing Nile tilapia with lysine + methionine increased significantly ($P<0.05$) DM contents of tilapia whole bodies. Also results of the same table revealed that regardless of amino acids supplementation increasing feeding frequency from 2 to 4 or 6 times daily resulted in significant ($P<0.05$) increase in DM contents of tilapia whole bodies. These results are not in accordance with results of Gomaa (1995) and Zaghloul (2005), who noticed that supplementing Nile tilapia diets with lysine or methionine did not released significant effects on tilapia DM contents. The same trend was observed with CP% contents, where T_6 showed significantly ($P<0.05$) the highest CP% contents followed in a significant decreasing order by T_5; T_3; T_4; T_2 and T_1, respectively (Table 5). The same table shows that supplementing Nile tilapia diets with lysine + methionine, improved significantly CP% contents in tilapia whole bodies regardless of feeding frequency. Also results of Table (5) revealed CP% contents in whole tilapia bodies in almost linear significant order with each increase in feeding frequency. Similar trend was also observed with EE%, thus EE contents of tilapia whole bodies increased significantly ($P<0.05$) with amino acids supplementation and feeding rate. Regarding the effects of feeding frequency on tilapia body composition, results of Table (5) are in accordance with the findings of Lee *et al.* (2000), who reported that as feeding frequency decreased moisture contents in muscles of Korean rockfish increased, protein contents were not affected and lipid contents in muscles; viscera and liver significantly increased.

Ash contents % in whole tilapia bodies showed the reverse trend compared to CP and EE, where amino acids supplementation and feeding frequency decreased significantly ($P<0.05$) ash content in whole tilapia bodies. Furthermore, results of Table (5) revealed that group T_6 showed the highest ($P<0.05$) body energy contents followed in a decreasing order by T_5; T_3; T_4; T_2 and T_1, respectively. As presented in the same table the main effects showed that lysine + methionine supplementation

Table (5) : Chemical composition of whole body of monosex Nile tilapia (*Oreochromis niloticus*) fed the experimental diets (% DM).

Diet		Nutrient Component (%)				
		DM	CP	EE	Ash	Energy (Kcal /Kg)
At the experiment start: Zero group		19.73^{g} ±0.09	51.42^{g} ± 0.50	24.32^{g} ± 0.03	23.21^{a} ± 0.01	5204.0^{g} ± 24.20
At the experiment end:						
Without supplemental amino acids	T_1	24.05^{f} ± 0.06	52.24^{f} ± 0.02	25.13^{f} ± 0.04	21.35^{b} ± 0.01	5326.07^{f} ± 0.84
	T_2	24.78^{e} ± 0.03	52.89^{e} ± 0.02	25.45^{e} ± 0.01	20.75^{d} ± 0.03	5393.03^{e} ± 1.54
	T_3	25.40^{c} ± 0.05	53.45^{c} ± 0.01	25.94^{c} ± 0.05	20.25^{e} ± 0.01	5470.78^{c} ± 4.45
With supple mental amino acids	T_4	25.27^{d} ± 0.01	53.02^{d} ± 0.03	25.89^{d} ± 0.00	21.22^{c} ± 0.01	5440.76^{d} ± 0.62
	T_5	25.79^{b} ± 0.02	53.64^{b} ± 0.05	26.12^{b} ± 0.02	20.07^{f} ± 0.02	5498.25^{b} ± 0.20
	T_6	26.17^{a} ± 0.03	54.95^{a} ± 0.04	26.75^{a} ± 0.02	18.15^{g} ± 0.01	5632.55^{a} ± 4.07
a, b, c etc: Means in the same column with different superscripts are significantly different (P< 0.05).						
Effect of supplemental amino acids	Without	24.74^{b} ± 0.07	52.86^{b} ± 0.07	25.51^{b} ± 0.05	20.78^{b} ± 0.06	5396.63^{b} ± 7.86
	With	25.74^{a} ± 0.05	53.87^{a} ± 0.08	26.25^{a} ± 0.05	19.81^{a} ± 0.17	5524.19^{a} ± 10.52
a, b, c etc: Means in the same column with different superscripts are significantly different (P< 0.05).						
Effect of feeding frequency	2	24.66^{c} ± 0.10	52.63^{c} ± 0.06	25.51^{c} ± 0.06	21.29^{b} ± 0.01	5383.92^{c} ± 9.31
	4	25.29^{b} ± 0.08	53.27^{b} ± 0.07	25.79^{b} ± 0.05	20.41^{c} ± 0.06	5445.64^{b} ± 8.48
	6	25.79^{a} ± 0.07	54.20^{a} ± 0.09	26.35^{a} ± 0.07	19.20^{a} ± 0.17	5551.67^{a} ± 13.29

a, b, c etc: Means in the same column with different superscripts are significantly different (P <0.05)

increased the energy contents of tilapia whole bodies. Also increasing feeding frequency from 2 to 4 or 6 times daily increased significantly ($p< 0.05$) energy contents in tilapia whole bodies, regardless of amino acid supplementation. Concerning the effect of lysine and methionine supplementation results of Table (5) are not in agreement with the findings of Gomaa (1995); Webster *et al.* (1992 a, b) and Zaghloul (2005), who reported that dietary supplementation of lysine and methionine to Nile tilapia diets released insignificant effects on body chemical composition.

Based on the results obtain in the present study it could be highly recommended to supplement the diets of Nile tilapia with amino acids lysine and methionine to reach 30% over the NRC (1993) recommended levels for better growth performance and nutrient utilization results. Also results may lead to recommend a feeding frequency 4 to 6 times daily for better growth performance and feed utilization of Nile tilapia.

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تأثير إضافة مستويات عالية من الليسين والميثيونين على أداء النمو لأسماك البلطي النيلى المغذاه على عليقة بعدد مرات مختلفة

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أجريت هذه الدراسة في محطة تجارب الاستزراع السمكي بقسم الإنتاج الحيواني - كلية الزراعة - جامعة الأزهر - القاهرة خلال الفترة من ٢٠٠٤/٧/١ إلى ٢٠٠٤/٩/٢٢.

تم توزيع عدد ٤٨٠ إصبعية بلطي نيلي وزن كل منه ١٠,٠٤ ± ٠٤, جرام في ستة مجموعات في مستويين (أحدهما بإضافة ٣٠٪ من الليسين والميثيونين فوق الاحتياجات والأخر بدون إضافة أي منهما) في كل من مستوى منهما ثلاث مستويات لعدد مرات التغذية (٢، ٤، ٦ مرة في اليوم).

أجريت التجربة في أحواض من الفيبر جلاس حجم كل منها ١م^3 تمد بالتهوية والمياه في دائرة مغلقة. وكانت المعاملات في أحواض مزدوجة في كل منها ٤٠ سمكة واستمرت التجربة لمدة ١٢ أسبوع، وكانت النتائج المتحصل عليها كالتالي:

- إضافة الليسين والميثيونين حتى ٣٠٪ فوق الاحتياجات حسن معنوياً الأوزان النهائية، والعائد من الوزن، البروتين المحتجز في الجسم، الطاقة المستفادة، ومن جهة أخرى وجد أن الإضافة ليس لها تأثير معنوي على معدل التحويل الغذائي ومعدل النمو النوعي.
- زيادة عدد مرات التغذية اليومية من ٢ إلى ٤ إلى ٦ مرات يومياً حسنت معنوياً الوزن النهائي للجسم والعائد من الوزن وأظهر تأثير معنوي على معدل التحويل الغذائي ومعدل النمو النوعي وقياسات كفاءة التغذية الأخرى.
- كلا من إضافة الأحماض الأمينية وعدد مرات التغذية أظهرت تأثير معنوي على التحليل الكيماوي لأجسام الأسماك.