REVIEWING THE OPTIMAL LEVEL OF METHIONINE AND LYSINE FOR TILPAIA FINGERLINGS (*OREOCHROMIS NILOTICUS*)

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Abstract

This study was carried out in the fish laboratory of the Central Laboratory for Food and Feeds - Agriculture Research Center and was designed to determine the optimal level of methionine and lysine in tilapia fingerling diets. Six experimental groups and a control treatment were assigned to one of the methionine or lysine levels. The control group contained methionine and lysine levels which were recommended by Santiago and Lovell (1988). All experimental diets were isonitrogenus (30% crude protein) and isocaloric (430 kcal / 100 g diet). Four dietary treatments with ascending levels of methionine (2.02, 2.72 (control), 3.45 and 4.11 g / 100 g crude protein), and varying levels of lysine (3.93, 5.12 (control), 6.2 and 7.87 g/ 100 g crude protein) were formulated. The seven tested diets were fed to 7 tilapia fingerling groups with initial body weight of 1.25 g / fish. Each experimental group consisted of 3 aquaria. The experiment duration was 14 weeks and the fish were fed at a rate of 3.5% of fish body weight daily. The daily allowances were fed at 5 meals. Results in terms of the highest final body weight, weight gain, specific growth rate, best feed conversion, protein efficiency and feed utilization showed that tilapia fingerlings require 2.72 g methionine and 5.12 g lysine / 100 g crude protein. Quadratic regression analysis of weight gain (%) against dietary methionine and lysine levels indicated that the optimal dietary methionine and lysine levels required for maximum growth was determined to be 3.05 % and 5.69 % of the dietary protein diet in the presence of 1.25 % cystine of the dietary protein diet, respectively. The results of the present study showed that the dietary requirements of methionine and lysine for tilapia fingerlings were 3.05% and 5.69% of the dietary crude protein, respectively.

INTRODUCTION

The available economic sources to fulfill the nutritional requirements of different commercial fish feed formulation in Egypt is now taking place to search for the most nutritive fish species. Protein sources are the most effective feed price, recently its price is tremendously increased. Therefore looking for the exact amino acids requirements is nowadays very essential. Plant proteins are less expensive than animal sources; however it's deficiency in methionine and lysine limiting its use as a main protein source in fish feeds. Many authors studied the requirements of different amino acids for tilapia. Jauncey *et al.* (1983) estimated the quantitative requirements of essential amino acids for *Oreochromis mossambicus.* Their estimated values were based on daily increase of essential amino acids in body protein, but without supportive growth data. Santiago and Lovell (1988) carried out a growth trial to quantify the requirements of essential amino acids of *Oreochromis niloticus* fry by using purified diets. The values given by Jauncey *et al.* (1983) were much lower than those given by Santiago and Lovell (1988). In commercial diets, both lysine and methionine appears to be the most limiting amino acids.

The aim of the present study was to revise the nutritional requirements of *Oreochromis niloticus* for lysine and methionine by using local commercial ingredients supplemented with varying levels of synthetic lysine and methionine.

MATERIALS AND METHODS

The present study was carried out during 21st April to 27th July 2005 at The Central Laboratory for Food and Feed (CLFF), Agricultural Research Center, Giza, Egypt. The experiment was conducted to determine the nutritional requirements of *Oreochromis niloticus* for lysine and methionine.

Culture conditions

Nile tilapia fingerlings were obtained from a local farm (Mazloum farm, Ismailia, Egypt). The fish were acclimated to laboratory condition for 4 weeks in fiberglass tanks. At the beginning of the experiment, 21 glass aquaria (60 L) were stocked each with 15 fish with an average initial weight of 1.25 g. The aquaria were supplied with continues flow fresh water (free of chlorine) at a rate of 2 L/min and were provided with supplemental aeration. The aquaria were illuminated using an overhead fluorescent lightning set on a 14:00 hour light: 10:00 hour dark cycle.

A thermo-controlled electric heater was used to adjust water temperature at the range of 24 - 27 °C. The dissolved oxygen and the nitrate values were found to be at the range of 5.1 - 6.2 mg/l and 20.0 - 40.2 mg/l respectively, while ammonia level was not detectable. The pH values were in the range of 6.8 - 7.7.

Diets and feeding regime

Seven experimental diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (30 %) and gross energy (430 kcal/ 100 g diet). Composition and the proximate analysis of the experimental diets are listed in Table (1) and their amino acid composition was listed in Table (2). Vitamin-mineral premix was obtained from Pfizer ® Company, Egypt Table (5).

These experimental diets were designed and formulated to contain different levels of either methionine (Met) or lysine (Lys) as follows:

Control (Diet 1): Contains the essential amino acid recommended for tilapia according to Santiago and Lovell (1988).

-25% Met. (Diet 2): Contains 25 % less than Methionine requirement for tilapia.

+25% Met. (Diet 3): Contains 25 % more than Methionine requirement for tilapia.

+50% Met. (Diet 4): Contains 50 % more than Methionine requirement for tilapia.

- 25% Lys. (Diet 5): Contains 25 % less than Lysine requirement for tilapia.

+25% Lys. (Diet 6): Contains 25% more than Lysine requirement for tilapia.

+50% Lys. (Diet 7): Contains 50% more than Lysine requirement for tilapia.

Chemical analysis

Analysis of the experimental formulated diets and whole fish body were carried out for moisture, nitrogen, ether extract, crude fiber and nitrogen free extract(NFE) according to the procedures of Association of Official Analytical Chemists (A.O.A.C, 1995) using triplicate samples for each determination, crude protein was calculated as nitrogen content x 6.25.

Determination of amino acids

Amino acids composition of the experimental diets are presented in Table 2. Amino acids except tryptophan and tyrosine were individually determined according to the methods of Official Journal of European Communities (1998). The system used for the analysis was high performance Amino Acid Analyzer, Beckman 7300.

The composition and the chemical analysis of the experimental diets are presented in Table (1). Feed ingredients were finely grinded and mixed manually, except DL- Methionine and Lysine-HCl. The amount of these amino acids were, first, dissolved in an amount of water before adding and then mixed with the respective diet. Water was added to each diet till paste was formed, and then passed through a meat mincer machine to convert the mixture into pellets. The wet pellets were then sun dried and stored at -20°C until used. Each diet was tested by three groups of 15 fish each. Fish fed five times a day, at a daily rate of 3.5% of fish body weight for 14 weeks. Every two weeks fish in each aquarium were weighed and the ration offered was changed according to their body weight. At the beginning of the experiment, one hundred fish were killed and kept frozen for chemical analysis. At the end of the experiment all fish in all aquaria were killed and kept frozen for chemical analysis.

Table 1. Composition and chemical analysis of the experimental diets used.

Ingredients	Diets						
	control	-25% Met	+25% Met	+50% Met	-25% Lys	+25% Lys	+50% Lys
Fish meal	5	5	5	5	5	5	5
Soybean meal	21	21	21	21	21	21	21
Gluten	21	21	21	21	21	21	21
Yellow corn	42.33	42.52	42.14	41.95	42.81	41.85	41.37
Fish oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Corn oil	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Premix (Vit & Min.)*	3	3	3	3	3	3	3
Mono calcium phosphate	1	1	1	1	1	1	1
DL Methionine	0.19	-	0.38	0.57	0.19	0.19	0.19
Lysine-Hcl	0.48	0.48	0.48	0.48	-	0.96	1.44
Total	100	100	100	100	100	100	100
Chemical composition							
Moisture %	7.50	7.10	6.70	6.80	7.30	7.10	7.50
Crude protein %	30.84	29.63	31.00	31.10	30.50	30.96	31.02
Ether extract %	8.10	8.15	8.20	8.10	8.00	8.10	8.14
Crude fiber %	2.90	2.91	2.95	3.00	2.88	2.92	2.89
Ash %	5.90	5.80	5.70	5.60	6.10	6.00	5.70
NFE*	44.76	46.41	45.45	45.40	45.22	44.92	44.75
GE*** (Kcal/l00g DM)	430	431	435	434	430	431	431

* Each kg contains: Vit A 4.8 mIU; D_3 0.8 mIU; E 4 g; K 0.8 g; B_1 0.4 g; B_2 1.6 g; B_6 0.6 g; B_{12} 4 g; Pantothenic acid 4g; Nicotinic acid 8 g; Folic acid 400 mg; Biotin 20 mg; Cholin chloride 299 g; copper 4 g; Iodine 0.4 g; Iron 12 g; Manganese 22 g; Zinc 22g and Selenium 0.04g.

** NFE = 100 - (Moisture + crude protein + ether extract + crude fiber+ Ash).

*** Gross energy (GE) content was calculated by using the factors 5.65, 9.45 and 4 Kcal/g for protein, ether extract and carbohydrate, respectively (Jobling, 1983)

Growth performance, and feed utilization parameters

Means of weight gain, percentage weight gain, average daily gain (ADG) and specific growth rate (SGR % day) were calculated according to the following equations:

1- Weight gain = $W1 - W_0$.
2- Percentage weight gain (WG %) = $(W_1 - W_0) / W_0 \times 100$.
3- Average daily gain (ADG) = weight gain/experimental period (d).
4- Specific growth rate (SGR %) = $\{(\ln W1 - \ln W_0)/T\} \times 100$.

Where:

W_0: Mean initial weight (g).

W_1: Mean final weight (g).

T: Time in days between weightings.

Means of feed conversion ratio (FCR), protein efficiency ratio (PER) and Protein Productive Value (PPV %) were calculated according to the following equations:

1- Feed conversion ratio (FCR) = Feed intake (g) / Weight gain (g).
2- Protein efficiency ratio (PER) = Weight gain (g). / Protein intake (g).
3- Protein Productive Value (PPV %) = $\{(BP_1 - BP_0)/CP\} \times 100$.

Where:

BP_0: Initial body protein content (g)

BP_1: Final body protein content (g)

CP: Protein intake (g)

Table 2. Essential amino acid analysis of the experimental diets (g/ 100 g crude protein)

Amino acid	Diets							Requirements*
	Control	-25% Met	+25% Met	+ 50% Met	-25% Lys	+ 25% Lys	+50 % Lys	
Therionine	3.76	3.78	3.74	3.79	3.83	3.83	3.78	3.75
Cysteine	1.25	1.30	1.20	1.18	1.20	1.18	1.20	1.18
Methionine	2.72	2.02	3.45	4.11	2.82	2.86	2.73	2.68
Isoleucine	3.92	3.85	3.82	3.78	3.96	3.70	3.91	3.11
Leucine	7.68	7.45	7.65	7.19	7.46	7.03	7.17	3.39
Phenylalanine	5.21	5.16	5.32	5.22	5.24	5.00	5.20	3.75
Valine	4.24	4.18	4.29	4.08	4.29	4.03	4.26	2.80
Lysine	5.12	5.16	5.16	5.14	3.93	6.20	7.87	5.12
Histdine	2.23	2.22	2.25	2.09	2.19	2.10	2.21	1.72
Arginine	4.22	4.36	4.32	4.38	4.24	4.33	4.17	4.20

Santiago and Lovell (1988)*

Water characteristics

Water temperature, pH, dissolved oxygen, ammonia NH_3 and nitrate NO_3 were all periodically measured during the feeding trials. Water temperature (°C) was measured by using a thermometer while pH was measured using ORION pH/ISE meter; model EA 940 EXPANDAPLE IONANALYZER according to Official Methods of Analysis (1993). Ammonia (NH_3) and dissolved oxygen were measured according to Standard Methods for the Examination of Water and Wastewater (1995a). ATI Orion ion meter was used with ammonium electrode model 95-12 and 97-80 for ammonia and oxygen measurements, respectively.

Statistical Analysis

The statistical analysis was computed by using analysis of variance procedure described by Snedecor and Cochran (1980) the significant mean differences between treatment means were separated by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Data representing means for initial weights, final weights, weight gain percentages, average daily gain and specific growth rates of fish given diets containing different levels of both methionine and lysine are presented in Table (3). The growth performance among the experimental fish groups were significantly different (P<0.05) for fish fed the control diet, where the highest final weight, weight gain, weight gain percentages, average daily gain and specific growth rate were achieved (5.95g, 4.72g, 382 %, 47 and 1.61 %, respectively). This observation indicated that the best performance of *Oreochromis niloticus* was obtained when fish were fed a diet containing the complete requirements of amino acids for tilapia according to Santiago and Lovell (1988).

Table 3. Growth performance of *Oreochromis niloticus* fish fed the 7 experimental diets

Item	Diets						
	Control	-25% Met	+25% Met	+50% Met	-25% Lys	+25% Lys	+50% Lys
initial weight (g/fish)	1.23^{a}	1.24^{a}	1.25^{a}	1.24^{a}	1.25^{a}	1.31^{a}	1.28^{a}
final weight (g/fish)	5.95^{a}	4.65^{cd}	5.13^{b}	5.06^{b}	4.53^{d}	5.07^{b}	4.84^{c}
Weight gain (g/fish)	4.72^{a}	3.41^{cd}	3.87^{b}	3.82^{b}	3.27^{d}	3.75^{b}	3.56^{c}
Weight gain (%)	382^{a}	275^{bc}	309^{b}	307^{b}	261^{c}	285^{b}	278^{bc}
Average daily gain (mg/fish/day)	47^{a}	34^{c}	39^{b}	38^{b}	33^{c}	38^{b}	36^{bc}
Specific growth rate (%/day)	1.61^{a}	1.34^{bc}	1.44^{b}	1.42^{b}	1.30^{c}	1.37^{bc}	1.35^{bc}

a, b, c, d = each raw with the same letter are not significantly different (p< 0.05).

Data in Table (4) representing means for feed conversion ratio (FCR), protein efficiency ratio (PER) and Protein Productive Value (PPV %) of fish given diets containing different levels of both methionine and lysine.

Table 4. Feed utilization parameters of *Oreochromis niloticus* fish fed the experimental diets.

DIETS							
Item	Control	-25% Met	+25% Met	+50% Met	-25 % Lys	+25 % Lys	+50% Lys
Feed conversion ratio (FCR)	1.64^{d}	2.25^{a}	2.02^{c}	2.10^{bc}	2.34^{a}	2.03^{c}	2.14^{b}
Protein efficiency ratio (PER)	1.98^{a}	1.49^{bc}	1.60^{b}	1.53^{bc}	1.39^{c}	1.61^{b}	1.51^{bc}
Protein Productive Value (PPV %)	26^{a}	18^{bc}	20^{b}	19^{b}	17^{c}	20^{b}	18^{bc}

a, b, c, d = each raw with the same letter are not significantly different ($p< 0.05$)

The best FCR, highest PER and the highest (PPV %) (1.64, 1.98 and 26, respectively) were achieved when fish fed the control diet which contained the recommended levels of amino acids for tilapia. (Lysine=5.12 % and methionine=2.72 % of dietary protein).

Compared to fish given the complete requirements of methionine and lysine (control diet) fish group given methionine deficient diet (-25% Met) showed higher feed conversion ratio (FCR), lower protein efficiency ratio (PER), and lower protein productive value (PPV %) (2.25, 1.49 and 18 %, respectively) when compare with the control treatment. The present findings are in agreement with Zhou *et al.* (2006) who reported that the lowest FCR for juvenile cobia (*Rachycentron canadum*) when fed on diet containing 1.05% methionine compared with the methionine levels (0.61, 0.83, 1.30, 1.48 and 1.68 %). PER increased with increasing dietary level of methionine from 0.61 % to 1.05% and decreased with further increase (1.68%**)**.

Luo *et al.* (2005) from their study on methionine requirement of juvenile grouper, they observed the poorest FCR (2.38), the lowest PER (0.93) were observed for fish fed the diet containing 0.55% methionine. These parameters improved proportionally with the methionine supplementation up to 1.34% and showed no significant differences for fish fed the dietary methionine level ranging from 1.34% to 1.81%. Also, Mai *et al.* (2006a) from their study on dietary methionine requirement of large yellow croaker, they found that the PER of fish were significantly improved by supplementation with methionine.

The highest PER (5.05) was recorded in the fish fed Diet 4containing 1.41% dietary methionine. PER significantly increased (from 2.77% to 5.05%) as the dietary methionine levels increased from 0.66% up to 1.41% of diets, and thereafter decreased with further increase in dietary methionine level from 1.41% to 1.89% dietary methionine levels.

Methionine deficiency resulted in reduced growth and feed efficiency, as well as in cataract of salmon (Walton *et al.*, 1982; Rumsey *et al.*, 1983; Cowey *et al.*, 1992).

Luo *et al.* (2005) from their study on methionine requirement of juvenile grouper, they observed low PPV % (16.3%) when fish fed the diets containing 0.55% methionine. Significant improvement in PPV % was observed when level of methionine supplementation increased at ranging from 1.34 to 1.81 % to be 28.8 and 31.6 % PPV %, respectively. Also, Kim *et al.* (1992b) observed in their study on rainbow trout, significant increase in nitrogen retention (19.2 to be 35.9%) with increasing the levels of methionine in the diets from (0.23% to 0.60%, respectively).

Compared to fish given the complete requirements of methionine and lysine (control diet) fish group given lysine deficient diet (-25% Lys) showed highest feed conversion ratio (FCR), lowest protein efficiency ratio (PER), and lowest protein productive value PPV % (2.34, 1.39 and 17 %, respectively). These results are in agreement with Ahmed and Khan (2004) who obtained best (FCR) and (PER) when they fed fish with 2.25% dietary lysine (break point = 2.38 % = 5.95 % of dietary crude protein). They also found that (PER) in fish fed a diet containing 2.25 % dietary lysine was significantly ($P<0.05$) higher than those fed other dietary lysine levels. Fish fed diets with higher lysine levels could not produce additional growth, whereas fish fed diets with lower lysine level showed lower weight gain and efficiency of feed utilization.

Furthermore, diets containing (+25% Lys or +50% Lys) over that of the control diet improved FCR performance of fish than that of the lysine deficient diet (-25 % Lys). These results are corresponding with that of Fagbenro *et al.* (1998a) who reported that African catfish fed ascending dietary lysine levels (40, 45, 50, 55, 60, 65 g/ kg protein) produced a significant increase ($P<0.05$) feed conversion up to 55 g/ kg protein then decreased (improved) feed conversion at level of 55-60 g lysine / kg protein. Supplementing diets with lysine beyond the level required for optimal growth may cause disproportionate levels of lysine and arginine (Kaushik and Fauconneau, 1984). Mai *et al.* (2006b) reported that PER values were reduced when juvenile Japanese sea bass fed lysine deficient diets (1.28 to 1.86%), while growth response and diet utilization were improved with supplementation of crystalline lysine at 2.46%. Wang *et al.* (2005) in their study on lysine requirement of juvenile grass carp found an improvement in protein efficiency ratio (PER) when increasing the levels of dietary lysine at 2.18% compared with 0.71, 1.2, and 1.69 %. of dietary lysine levels also, they found that the excess of lysine levels at 2.67 and 3.16 % of dietary lysine decreased the (PER).

Protein productive value (PPV %) observed in the present study with the control diets was higher than the other experimental groups. Our observations are in agreement with Ahmed and Khan (2004) who found that protein deposition in Indian major carp fed diet with 2.25 % (optimum level) lysine was found to be significantly higher (improved) compared with other dietary lysine levels 1.5, 1.75, 2.0, 2.5, and 2.75 %. Also, our results are in agreements with Wang *et al.* (2005) in their study on lysine requirement of juvenile grass carp that an improvement in (PPV %) when increasing the levels of dietary lysine level at 2.18 % compared with 0.71, 1.2, and 1.69 %. Also, they found that the excess of lysine levels at 2.67 and 3.16% decreased the (PPV %).

Berge *et al.* (1998) these authors determined the dietary lysine requirement of Atlantic salmon and found that fish fed diets containing low levels of lysine showed reduced feed utilization. Increasing lysine level in the diet improved the protein efficiency ratio (PER) and the feed conversion ratio (FCR). Higher level of lysine than the suggested requirement did not improve feed utilization.

Data in Table (5) Representing means for Body moisture, crude protein, ether extract and ash content of fish given diets containing different levels of both methionine and lysine.

Table 5. Chemical composition of *Oreochromis niloticus* fish fed the 7 experimental diets

Item	Diets						
	CONTROL	-25% Met	+25% Met	+50% Met	-25% Lys	+25% Lys	+50% Lys
Moisture %	74.70^{c}	76.29^{ab}	75.73^{b}	75.96^{ab}	76.28^{ab}	76.50^{a}	76.60^{a}
Crude protein %	52.49^{a}	51.43^{a}	51.97^{a}	52.55^{a}	51.7^{a}	52.49^{a}	52.57^{a}
Ether extract %	27.18^{b}	28.05^{a}	27.22^{b}	27.16^{b}	27.98^{a}	27.37^{b}	27.23^{b}
Ash %	10.23^{bc}	9.93^{c}	11.03^{ab}	10.17^{bc}	11.20^{a}	10.83^{abc}	10.8^{abc}

a, b, c = each raw with the same letter are not significantly different ($p< 0.05$)

Body moisture, crude protein, ether extract and ash content of fish given four ascending levels of methionine (2.02, 2.72 (control), 3.45 and 4.11 g / 100 g crude protein).are shown in (Table 11 and Fig 10, 11, 12, 13)

Body moisture content of fish given 2.02% methionine (g/100g crude protein) (-25 % Met) was significantly ($P < 0.05$) higher than that fish given 2.72 % methionine (g/100g crude protein) (control diet).

However, body moisture content of fish given 2.02% methionine (g / 100g crude protein) (-25% Met) was not significantly different ($p > 0.05$) from those given the other methionine level (3.45g methionine and 4.11g methionine g/100g crude protein).

Also body moisture content of fish given 2.72% methionine (g/100g crude protein) (control) was significantly ($P < 0.05$) lower than from those given the other methionine level (3.45g methionine and 4.11 methionine g / 100g crude protein). The results showed that the body moisture content of fish fed different levels of dietary methionine decreased ($P < 0.05$) with increasing methionine levels up to 2.72% g/100g crude protein (control diet) and thereafter an increase in body moisture content with increasing dietary methionine levels. Although body crude protein content was not significantly different ($p > 0.05$) among fish given ascending levels of methionine (2.02, 2.72 control), 3.45 and 4.11 g / 100 g crude protein), body crude protein increase with increasing dietary methionine levels.

The body ether extract (EE) of fish given 2.02% methionine (g/100g crude protein) (- 25% Met) was significantly ($P < 0.05$) higher than from those given the other methionine levels. The result showed that (EE) content decrease with increasing dietary methionine levels.

Ash content was found to be not significantly different ($p > 0.05$) among fish given ascending levels of methionine (2.02, 2.72 control diet), 3.45 and 4.11 g / 100 g crude protein), the lowest content of ash was recorded for fish given 2.02% methionine (g / 100g crude protein) (-.25% Met) while the highest was recorded for fish given 4.11% methionine (g/100g crude protein) +50% Met). Schwarz *et al.* (1998) in their study on carp *(Cyprinus carpio L)* found that whole body protein and lipid content were significantly affected by dietary methionine levels ($P < 0.01$). The whole body protein increased significantly from 14.7% T-I to 16.3% T-IV with increasing dietary methionine levels, while fat decreased from 13.9 T-I to 11.5% T-V with increasing dietary methionine levels, (Where T-I to T-V are treatments with ascending methionine levels 0.49, 0.61, 0.79, 1.08,and 1.34%).

Luo *et al.,* (2005) from their study on methionine requirement of juvenile grouper, they observed that carcass protein content showed an increasing trend with increasing dietary methionine levels ($P < 0.05$) but moisture content decreased ($P < 0.05$). Ash content kept relatively constant among these treatments.

Body moisture, crude protein, ether extract and ash content of fish given four ascending levels of lysine (3.93, 5.12 (control diet), 6.2 and 7.87 g/ 100 g crude protein), are shown in (Table 11 and Fig 14, 15, 16, 17).

Body moisture content of fish given 3.93% lysine (g/100g crude protein) (-25 % Lys) was significantly ($p < 0.05$) higher than that fish given 5.12% lysine (g/100g crude protein) (control). However, body moisture content of fish given 3.93% lysine (g/100g crude protein) (-25 % Lys) was not significantly affected ($p > 0.05$) from those given the other lysine level (3.45g lysine and 4.11g lysine / 100g crude protein).

Also body moisture content of fish given 5.12% lysine (g/100g crude protein) (control) was significantly ($P<0.05$) lower than from those given the other lysine levels (3.45 lysine and 4.11 g lysine / 100g crude protein). The result showed that the moisture content of fish fed different levels of dietary lysine decreased ($P < 0.05$) with increasing lysine level up to 5.12% g/100g crude protein (control diet) and thereafter an increase in moisture with increasing dietary lysine levels. Body crude protein content was not significantly difference ($P >0.05$) among fish given ascending levels of lysine (3.93, 5.12 (control diet), 6.2 and 7.87 g / 100 g crude protein), body crude protein increase with increasing dietary lysine levels.

The body ether extract (EE) of fish given 3.93% lysine (g/100g crude protein) (- 0.25 % Lys) was significantly ($P< 0.05$) higher than those given the other lysine levels. The result showed that (EE) content decrease with increasing dietary lysine levels. Ash content was not significantly different ($p>0.05$) among fish given ascending levels of lysine (3.93, 5.12 (control), 6.2 and 7.87 g / 100 g crude protein).

Our observations are in agreement with Ahmed and Khan (2004) in their study on lysine requirement in Indian major carp. The moisture content in the carcass composition of fish fed different levels of dietary lysine decreased ($P < 0.05$) with increasing dietary lysine level up to 2.25% and thereafter an increase in moisture was noted. Mai *et al.* (2006b) found in their study on juvenile Japanese sea bass that, the whole body protein and lipid were significantly affected by dietary lysine levels ($P < 0.01$). The whole body protein (range from 18.8% to 21.2%) was positively correlated with dietary lysine level, while lipid (range from 8.4% to 7.3%) was negatively correlated with it.

Rodehutscord *et al.* (2000) found that the crude protein (CP) in trout body increased linearly with increased dietary lysine supplementation disregarding dietary CP levels, and fat decreased linearly with increased lysine supplementation.

Wang *et al.* (2005) in their study on lysine requirement of juvenile grass carp that minimum body moisture content was observed in fish fed 2.18 % dietary lysine. Compared with 0.71, 1.2, 1.69, 2.67 and 3.16 % of dietary lysine. Also, they found that moisture content of whole body decreased with increasing dietary lysine levels up to 2.18% .thereafter increased with further increase in dietary lysine level

Cheng *et al.* (2003) from their study on rainbow trout (*Oncorhynchus mykiss*) found that the whole body moisture content, CP, fat and ash among fish fed different diets. Lysine supplementation in the plant protein-based diets increased CP and reduced fat in fish body. An indispensable amino acid deficiency may cause reduced growth and poor feed conversion (Wilson and Halver, 1986), therefore, satisfying the indispensable amino acid requirements of a species is outmost importance in preparing well-balanced diets.

Data representing means for results of growth and feed utilization of the Nile tilapia *Oreochromis niloticus,* fed different levels of methionine are showed in Table (9 and 10). Dietary methionine level significantly affected growth and feed utilization ($P < 0.05$). Weight gain and SGR increased with increasing dietary methionine level from 2.02% to 2.72% ($P < 0.05$); however, with further increase in dietary methionine level from 2.72% to 4.11%, these parameters decreased. The relationship curve equation between weight gain (%) and dietary methionine levels was best expressed by the broken-line regression model (at a constant dietary cystine level of 1.2%) $y = -57.97x2 +358.38x +200.09$ ($R2 = 0.5065$) (Fig. 1). and the breakpoint for WG occurred at 3.05% dietary methionine. The optimal dietary methionine levels requirement based on maximum Weight gain (%) was determined to be 3.05% of the dietary protein diet in the presence of 1.25% cystine of the dietary protein.

These results are similar to those in other fish species, such as common carp 3.1% of dietary protein (Nose, 1979), Asian sea bass 2.9% of dietary protein (Coloso *et al.*, 1999), Atlantic salmon 3.1% of dietary protein (Rollin, 1999), red drum 3.0% of dietary protein (Moon and Gatlin, 1991), Indian major carp (*Cirrhinus mrigala*) 3.0% of dietary protein (Ahmed *et al.*, 2003), African catfish 3.2% of dietary protein (Fagbenro *et al.*, 1998b) and Indian major carp (*Labeo rohita* Hamilton) 3.23% of dietary protein (Murthy and Varghese, 1998) and lower than that for catla 3.6% of dietary protein (Ravi and Devaraj, 1991), but exceeded those reported for coho salmon 2.7% of dietary protein (Arai and Ogata, 1993), yellowtail 2.6% of dietary protein (Ruchimat *et al.*, 1997b), rohu 2.6% of dietary protein (Khan and Jafri, 1993), grouper 2.73% of dietary protein (Luo *et al.*, 2005), Japanese flounder 1.9% of dietary protein (Alam *et al.*, 2001), rainbow trout 2.3% of dietary protein (Kim *et al.*, 1992b; Rodehutscord *et al.*, 1995), milkfish 2.5% of dietary protein (Borlongan and Coloso, 1993), European sea bass 2.0% of dietary protein (Thebault *et al.*, 1985) and Arctic charr 1.8% of dietary protein (Simmons *et al.*, 1999).

Data representing means for results of growth and feed utilization of the Nile tilapia *Oreochromis niloticus,* fed different levels of lysine are shown in Table (9 and 10). Dietary lysine level significantly affected growth and feed utilization ($P<0.05$). Weight gain and SGR increased with increasing dietary lysine level from 3.93% to 5.12% g / 100g crude protein ($P<0.05$); however, with further increase in dietary lysine level from 5.12% to 7.87%, these parameters decreased. The relationship curve equation between weight gain (%) and dietary lysine levels was best expressed by the broken-line regression model $y = -15.799x2 +183.42x - 197.65$ ($R2 = 0.3663$) (Fig. 2). and the breakpoint for WG occurred at 5.69 % dietary lysine. The optimal dietary lysine levels requirement based on maximum Weight gain (%) was determined to be 5.69 % of the dietary crude protein.

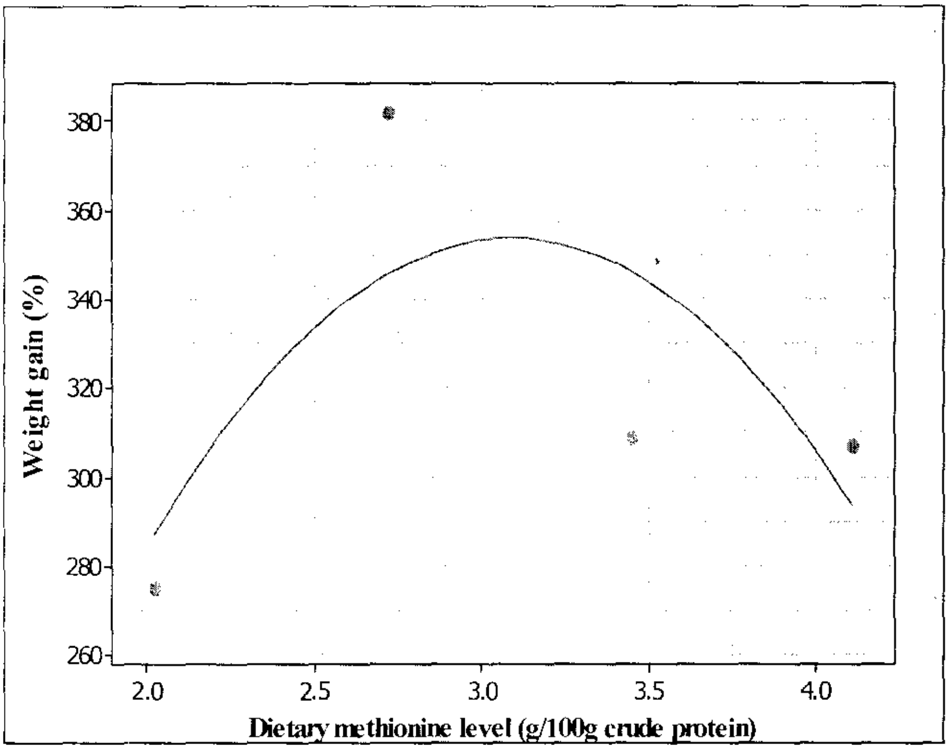

Fig. 1. Relationship between WG % and dietary methionine levels for *Oreochromis niloticus* juveniles based on broken-line regression analysis, where X axis represents the optimal dietary methionine level for the maximum WG% of grouper.

This value is similar to values reported for certain other species, such as common carp 5.70% (Nose, 1979), red drum 5.7% (Brown *et al.*, 1988), rohu 5.88% (Khan and Jafri, 1993), 5.68% (Murthy and Varghese, 1997), African catfish 5.7% (Fagbenro *et al.*, 1998a) and C. mrigalared 5.75% (Ahmed and Khan, 2004) but is higher than those reported for rainbow trout 3.7% (Kim *et al.*, 1992a), and for coho salmon, 3.8% (Arai and Ogata, 1991), and lower than that for catla 6.2% (Ravi and Devaraj, 1991), or rainbow trout 6.1% (Ketola, 1983) and Atlantic salmon 6.1% (Rollin, 1999). The wide variation observed in the requirements for lysine among fish species may be due to the differences in dietary protein sources, the reference protein which amino acid pattern is being imitated (Forster and Ogata, 1998), diet formulation, size and age of fish, genetic differences, feeding practices and rearing conditions (Ruchimat *et al.*, 1997a).

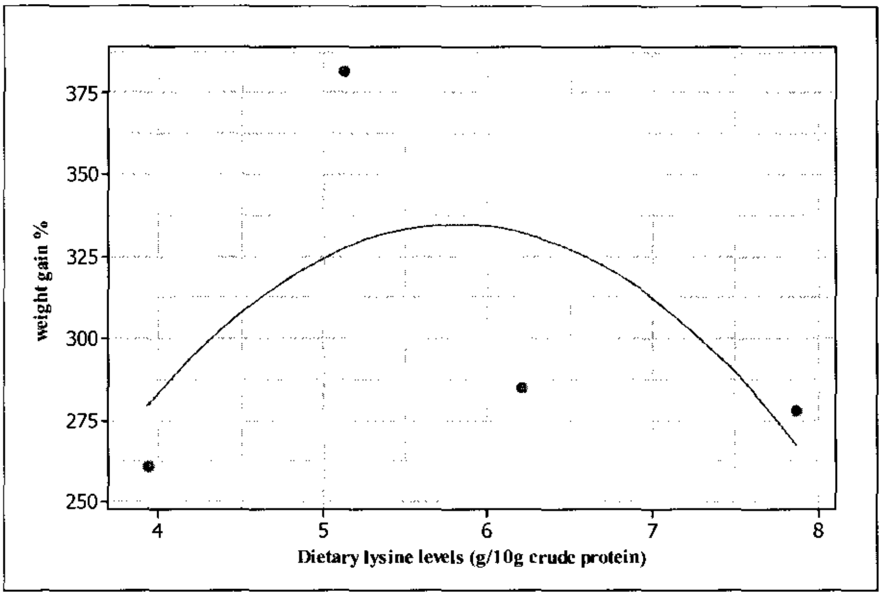

Fig. 2. Relationship between WG % and dietary lysine levels for *Oreochromis niloticus* juveniles based on broken-line regression analysis, where represents the optimal dietary lysine levels for the maximum WG% of grouper.

CONCLUSION

This observations of the presents study indicated that under the present experimental conditions the best performance parameters were obtained by feeding tilapia fingerlings diets containing 5.12% lysine and 2.72% methionine of the crude protein; however, the regression analysis showed that the requirements of lysine and methionine were 5.69% and 3.05% of the crude protein, respectively.

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