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Lipid utilization, protein sparing effects and protein requirement of all male Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in underground brackish water

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Abstract

Nile tilapia *Oreochromis niloticus* as a species with high growth rate and high tolerate to environmental conditions and stocking density, have been culturing in the world more and more. Because of feed expenses necessity and better growth rate and feed conversion efficiency in brackish water, despite of existing many studies on protein requirements, lipid utilization and protein sparing effect of Nile tilapia, they were studied on all males in brackish water, again. A 4 × 3 factorial design with three replicates was planned based on the proximate and amino acids analysis of protein sources to contain four crude protein (15, 22, 29 and 36 %) and three crude lipid (5, 9 and 13%) levels. Growth indices including weight gain and specific growth rate improved by protein accretion and reduced by lipid increasing. The best growth performance was obtained in 5% crude lipid. Feed conversion ratio reduced by protein increasing, and the best one observed in the diet containing 36% crude protein. Protein efficiency ratio and Protein conversion efficiency increased by protein accretion up to 29% crude protein. Protein efficiency, except 15% crude protein, increased up to 9% crude lipid and then reduced but not significantly; so, protein-sparing effect appeared, unclearly. In this study, at 25.6% crude protein with 265 mg protein per gram body weight, fish can keep their carcass protein percentage. In economic point of view, based on growth, feed and protein performances, the levels of 29% crude protein and 5% crude lipid are sufficient for convincing growth rate. The results show that isotonic environment cannot Change protein and lipid requirements compared to the other studies in the fresh water.

Keywords: Protein, Lipid, Tilapia, Brackish water

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Introduction

Nile tilapia *Oreochromis niloticus* is one of the most important species in the world aquaculture industry. It has some good characteristics including high growth rate, eurythermality, simple artificial reproduction; low feed requirements that cause high cheap yield, artificial feed acceptance immediately after yolk suck absorbance and taste. Therefore, Nile tilapia has been come the best aquaculture species in developing countries (El-Saidy and Gaber, 2005). It was introduced to the National Research Center of Saline Water Aquatics, Iran with the aim of culturing in central states of Iran in underground brackish water.

Since, the energetic cost of osmoregulation is lower in an isosmotic medium, where the gradients between blood and water are minimal; most of the fishes, both marine and fresh water, have lowest standard metabolic rates, so growth and food conversion efficiency are clearly influenced and would be better (Boeuf and Payan, 2001). According to this and controlling feed intake by fish (Le Bail and Boeuf, 1997), it seems that protein and lipid requirements are different and lower in brackish water. So, feed as the most economical factor, protein as the most expensive nutrient and lipid that makes protein sparing should be attended.

Lipid affects growth, feed and protein performances; so, probably induce protein sparing more than carbohydrates. In tilapia diets, lipids for maximum growth is generally about 10-15%; But, farmers use lower levels between 6-8% (El-Sayed, 2006). In the fish diets, if lipid becomes more or less, it has negative effects on growth rate (El-Sayed and Teshima, 1992; Gumus and Ikiz, 2009). Fish use lipids until specific level; growth decreases after and before that because of less feed intake, and de-ammunition and protein catabolism, respectively (Page and Andrews, 1973; Winfree and Stickney, 1981; Watanabe, 1982; Ellis and Reigh, 1991; Kaushik and Medale, 1994; Wang *et al*., 2005; El-Sayed and Kawanna, 2008).

Proteins and lipids of finfishe feed depend on species, size, age, life stage, the environment and sexuality are different (El-Sayed 2006). They were studied extensively (NRC, 1993) in tilapia and the protein requirement has been reported about 30 percent; but it changes according to the location and environmental conditions. Therefore, according to necessity of understanding optimum protein requirement and lipid utilization of all male Nile tilapia in brackish water and economical point of view, this study was planned.

Materials and methods

Experimental diets

A 4 × 3 factorial design with three replicates was planned in this study. According to the proximate and amino acids analysis of protein sources, twelve experimental diets were formulated by Lingo (version 14) containing four protein levels (15, 22, 29 and 36 %) and three lipid levels (5, 9 and 13%) that supply minimum essential amino acids requirements based on Santiago and Lovell (1988). Diets (3 mm pellets) were named as 15:5, 15:9, 15:13, 22:5,

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22:9, 22:13, 29:5, 29:9, 29:13, 36:5, 36:9 and 36:13 (CP%:CL%), respectively. Formulation and proximate composition of the experimental diets are presented in Table 1. Fishmeal and Soybean meal served as main protein sources and dietary protein level was increased by adjusting the amount of fishmeal and soybean meal at equal proportions. Soybean oil has been used as the single lipid source for energy.

Table 1: Formulation and proximate composition of the experimental diets.

Ingredients (%)	diets											
	15:5	**15:9**	**15:13**	**22:5**	**22:9**	**22:13**	**29:5**	**29:9**	**29:13**	**36:5**	**36:9**	**36:13**
Fish meal	7.58	7.62	7.65	13.97	14.01	14.05	20.37	20.40	20.44	27.50	27.53	27.57
Soybean meal	13.91	13.87	13.84	18.97	18.94	18.91	24.04	24.01	23.98	27.83	27.80	27.77
Wheat flour	30	30	30	30	30	30	30	30	30	30	30	30
Vitamin C [1]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Vitamin complex [2]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Mineral complex [3]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Threonine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Choline	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Corn starch	44.71	41.13	37.55	34.07	30.49	26.91	23.43	19.85	16.28	12.94	9.67	6.10
Soybean oil	2.12	5.69	9.26	1.30	4.87	8.44	0.47	4.04	7.62	0.04	3.31	6.88
Sum	100	100	100	100	100	100	100	100	100	100	100	100
Crude protein	15.76	15.56	15.75	22.52	22.70	21.96	28.60	28.70	28.64	35.62	35.23	35.51
Ether extract	5.44	9.31	13.36	5.73	9.30	13.01	6.04	10.37	13.79	5.98	9.22	13.23
Gross energy [4]	4.48	4.69	4.89	4.54	4.74	4.92	4.57	4.81	4.99	4.60	4.78	5.01
Crude fiber	1.09	1.02	1.44	1.37	1.37	1.29	1.77	1.80	1.79	2.01	1.94	1.76
Ash	2.67	2.62	2.67	3.93	3.79	3.96	5.32	5.29	5.29	6.97	6.74	6.63
Moisture	9.10	8.53	7.90	9.20	9.25	8.15	9.04	8.46	8.29	9.15	8.16	7.56
P/E ratio [5]	35.19	33.20	32.22	49.64	47.93	44.65	62.53	59.70	57.39	77.39	73.68	70.84

1- Vitamin C purity = 50%
2- Vitamin A 30000000 IU L^{-1}; D3 8000000 IU L^{-1}; E 16000 IU L^{-1}; B1 2000 mg L^{-1}; B2 2000 mg L^{-1}; B6 2000 mg L^{-1}; Nicotinamide 2000 mg L^{-1}; Vitamin C 10000 mg L^{-1}; K3 2000 mg L^{-1}; Biotin 20 mg L^{-1}; D-Panthenol 5000 mg L^{-1}; Lysine 30000 mg L^{-1}; Methionine 20000 mg L^{-1}.
3- Zn 40000 mg kg^{-1}; Mn 20000 mg kg^{-1}; Cu 5000 mg kg^{-1}; Fe 5000 mg kg^{-1}; Se 300 mg kg^{-1}.
4- Kcal g^{-1}
5- Mg $Kcal^{-1}$

All diets were prepared in the laboratory. Ingredients were ground into fine powder and mixed thoroughly with soybean oil in a feed mixer. An appropriate amount of water has added to produce stiff dough. Then, the dough was passed through a mincer and dried in a drying machine at 60 °C for 18 h. After drying, the diets were broken up and sieved into proper pellet size. All diets were stored at -20 °C in the plastic-lined bags until used.

Experimental fish and feeding trial

All male Nile tilapia was prepared in the National Research Center of Saline Water Aquatics (Yazd province, Iran) by hormonal methods. Prior to the experiment, fish had been reared in tanks for 2 weeks to acclimate to the experimental conditions and diets. At the first week, they fed by commercial rainbow trout diet. After the acclimation, fish with similar sizes (initial weight=13.94±0.88 g) were randomly distributed into 36 under aeration 150 L polyethylene tanks, each

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15 fish were stocked in a flow-through system at a flow-rate of 3 L min^{-1} underground brackish water at the salinity of 8 g L^{-1}. A 12-h light: 12-h dark cycle of illumination was provided by fluorescent lights. Fish were hand-fed to apparent satiation two times daily (08:00 and 14:00 h) for 8 weeks. Extra feed collected 30 min after each feeding. Temperature, dissolved oxygen (DO) and pH were monitored daily; and unionized ammonia and nitrite weekly were measured.

Sample collection and chemical analysis

At the first and the end of the feeding trial, ten fish were anesthetized with 150-200 mg L^{-1} clove oil, their viscera removed and have been stored at -20 °C for subsequent analysis. All fish of every tank caught and anesthetized for analysis of body weight, biweekly.

Ingredients (fishmeal, soybean meal, and wheat meal and cornstarch), diet and fish were analyzed for proximate composition according to AOAC (2005). Moisture was determined by oven drying until constant weight (105 °C). Crude protein was determined by the Kjeldahl method (BEHR Kjeldahl system, Germany); crude lipid by Soxhlet ether-extraction (BEHR, Germany); crude fiber by glass crucible method (Fibertec. system, Italy); ash by combustion at 550 °C for 4 h in a Muffle furnace; Nitrogen-free extract (NFE) was calculated by difference; and gross energy was calculated by using conversion factors of 9.44, 5.65 and 4.1 $Kcal/g^{1}$ for lipid, protein and NFE, respectively (Einen and Roem, 1997). Essential amino acids of the protein sources were analyzed by HPLC.

Statistical analysis

Data was analyzed by using the SPSS (version 16, USA) for significant differences among treatments means based on the amount of protein, lipid, and the interaction of protein and lipid. If significant (p<0.01) differences were found in factors, Duncan's multiple range test has used to rank the means. All data are presented as Means±S.D. (standard division of the mean) of three replications.

Results

Water temperature was fixed at 27 °C (27.27±0.08) by central heater. DO and pH were 5.72±0.06 and 7.1±0.15, respectively. Unionized ammonia and nitrite fluctuated 0.0023±0.00008 mg L^{-1} and >0.02 mg L^{-1}, respectively. Survival was 100% in all treatments except 22:5, 29:9, 29:13 and 36:5 (97.7%).

Specific growth rate (SGR) and weight gain (WG) were gone up significantly (p<0.01) by protein increasing until 29% crude protein level. WG decreased in 9 and 13% crude lipid levels, significantly (p<001; Table 2).

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Table 2: Growth, food and protein performance and carcass proximate composition of tilapia fed various protein and lipid levels*.

indices	diets											
	15:5	15:9	15:13	22:5	22:9	22:13	29:5	29:9	29:13	36:5	36:9	36:13
Initial weight (g)	13.27±0.85^{a}	13.77±1.04^{a}	13.29±0.87^{a}	14.31±1.16^{a}	14.58±0.80^{a}	14.40±0.49^{a}	14.43±1.27^{a}	14.87±0.37^{a}	13.84±1.02^{a}	13.40±0.54^{a}	13.84±0.81^{a}	14.22±0.30^{a}
Final weight (g)	36.57±2.26	32.60±2.37	29.63±3.47	57.69±1.28	52.17±0.72	43.09±4.00	67.47±8.73	61.60±0.43	58.28±3.24	66.34±2.21	68.40±7.55	57.94±2.39
WG[1] (%)	176.2±24.5^{ab}	136.8±4.9^{ab}	122.7±16.6^{a}	304.4±23.2^{de}	258.2±14.6^{cd}	199.7±33.8^{bc}	368.0±51.7^{ef}	314.4±13.3^{de}	322.4±36.9^{de}	395.1±10.7^{f}	396.9±80.2^{f}	307.7±23.5^{de}
SGR[2] (%/day)	1.81±0.16^{b}	1.54±0.04^{a}	1.43±0.14^{a}	2.49±0.10^{cd}	2.28±0.07^{c}	1.95±0.20^{b}	2.75±0.19^{de}	2.54±0.06^{cd}	2.57±0.16^{cde}	2.86±0.04^{e}	2.85±0.28^{e}	2.51±0.10^{cd}
FCR[3]	2.71±0.17^{d}	3.06±0.13^{e}	3.67±0.32^{f}	1.74±0.03^{c}	1.76±0.00^{c}	1.91±0.16^{c}	1.35±0.09^{b}	1.32±0.01^{ab}	1.33±0.07^{ab}	1.12±0.04^{ab}	1.08±0.04^{a}	1.16±0.07^{ab}
TFI (g)	945.5±35.3^{cd}	863.1±35.5^{abc}	889.9±75.3^{bcd}	1106.6±46.9^{f}	991.5±0.3^{de}	815.0±50.5^{ab}	1067.8±104.7^{ef}	936.4±29.6^{cd}	870.3±53.7^{abc}	867.5±31.4^{abc}	881.9±103.9^{bcd}	762.7±23.3^{a}
PI (g)	135.4±5.06^{a}	122.8±5.20^{a}	129.1±10.92^{a}	226.3±9.60^{cd}	204.2±0.07^{c}	164.4±10.19^{b}	277.8±27.25^{e}	246.0±7.78^{d}	228.6±14.11^{cd}	280.7±10.15^{e}	285.4±33.62^{e}	250.3±7.64^{d}
EI (Kcal)	3850.2±143^{abc}	3701.6±156^{ab}	4006.7±339^{abcd}	4558.7±193^{e}	4261.5±1.55^{cde}	3682.8±228^{ab}	4442.7±435de	4120.4±130^{bcde}	3982.4±245^{abcd}	3627.3±131^{ab}	3872.7±456^{abc}	3533.8±108^{a}
PER[4]	2.57±0.17^{c}	2.30±0.10^{b}	1.89±0.17^{a}	2.81±0.06^{cd}	2.76±0.01^{cd}	2.61±0.22^{cd}	2.85±0.17^{cd}	2.89±0.01^{d}	2.86±0.14^{d}	2.76±0.11^{cd}	2.86±0.11^{d}	2.62±0.15^{cd}
PCE[5](%)	35.66±3.01^{b}	31.93±4.01^{b}	25.90±2.53^{a}	41.37±1.14^{c} ^{d}	42.08±1.44^{d} ^{e}	36.83±4.17^{b} ^{c}	48.28±2.62^{f}	48.89±0.06^{f}	47.24±3.39^{ef}	47.81±0.36^{f}	48.29±3.23^{f}	47.28±2.84^{ef}
CPI[6] (%)	-7.76	-7.02	-7.24	-5.09	-4.77	-7.07	5.56	4.91	3.32	7.59	5.39	10.74
Crud Protein[7] (%)	14.57±0.59^{a}	14.69±0.66^{a}	14.65±0.24^{a}	14.99±0.11^{a}	15.04±0.66^{a}	14.68±0.26^{a}	16.67±0.32^{b}	16.57±0.15^{b}	16.32±0.29^{b}	16.99±0.44^{bc}	16.65±0.50^{b}	17.49±0.34^{c}
Crud lipid[7] (%)	9.82±1.12^{cd}	11.01±1.26^{d}	10.47±0.44^{cd}	9.42±1.07^{c}	9.42±0.43^{c}	10.99±0.32^{d}	6.05±0.36^{a}	7.98±0.55^{b}	9.26±0.32^{c}	5.56±0.55^{a}	7.42±0.79^{b}	7.39±0.39^{b}

- Values are means ± SD of 3 replications. Means in the same rows with different superscripts are significantly different (p<0.01).

1- Weight gain = (final body weight – initial body weight)×100/initial body weight.

2- Specific growth rate (SGR)=(Ln final weight–Ln initial weight)×100/culture period in days.

3- Food conversion ratio (FCR)=total food intake/total wet weight gain.

4- Protein efficiency ratio (PER)=Fish wet weight gain/protein intake.

5- Protein conversion efficiency (PCE)=$[(W_f \times P_f)–(W_i \times P_i)] \times 100 / (P_d \times TFI)$, where W_i and W_f are the initial and final body weights, P_i and P_f are the initial and final protein contents in carcass, respectively, and P_d is the protein content in the diets.

6- Carcass protein increasing (CPI) = (final carcass protein percentage – initial carcass protein percentage) × 100/initial carcass protein percentage.

7- According to wet weight

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There was an interaction between protein and lipid levels in food conversion ratio (FCR) (p<0.01); it increased significantly (p<0.01) by lipid enhancement in 15% protein, while in the upper protein levels (29 and 36%) this growth accretion did not happened (p>0.01; Table 2). The synchronous increasing and decreasing of SGR and FCR until 29% protein were found by protein enhancement respectively.

Total feed intake first enhanced then reduced by protein increasing, significantly (p<0.01). And there were not any significant differences between these two pair of crude protein levels 15 with 36 and 22 with 29 % (Table 2). Protein accretion and lipid reduction increased protein intake. In the upper protein levels (29 and 36 %CP), protein intake—has been reduced by lipid increasing more than 5%. Higher energy intake had happened in the middle of protein levels (22 and 29 %CP) in diets with 5% crude lipid; at the other lipid levels, energy intake resembled in all protein levels (Table 2).

A significant accretion and reduction in protein efficiency ratio (PER) and protein conversion efficiency (PCE) happened with protein and lipid increasing, respectively (p<0.01); except within 29 and 36 %CP in all lipid levels (Table 2).

Carcass protein enhanced by protein increasing, but carcass lipid reduced, inversely. Carcass lipid increased by lipid enhancement, neither carcass protein. Carcass protein increasing (CPI) was below zero in 15 and 22% protein and increased by protein accretion. If CPI equal zero, carcass protein percentage, protein intake and final mass were 25.6%, 225.6 g and 851.5 g, respectively (Figs. 1 and 2).

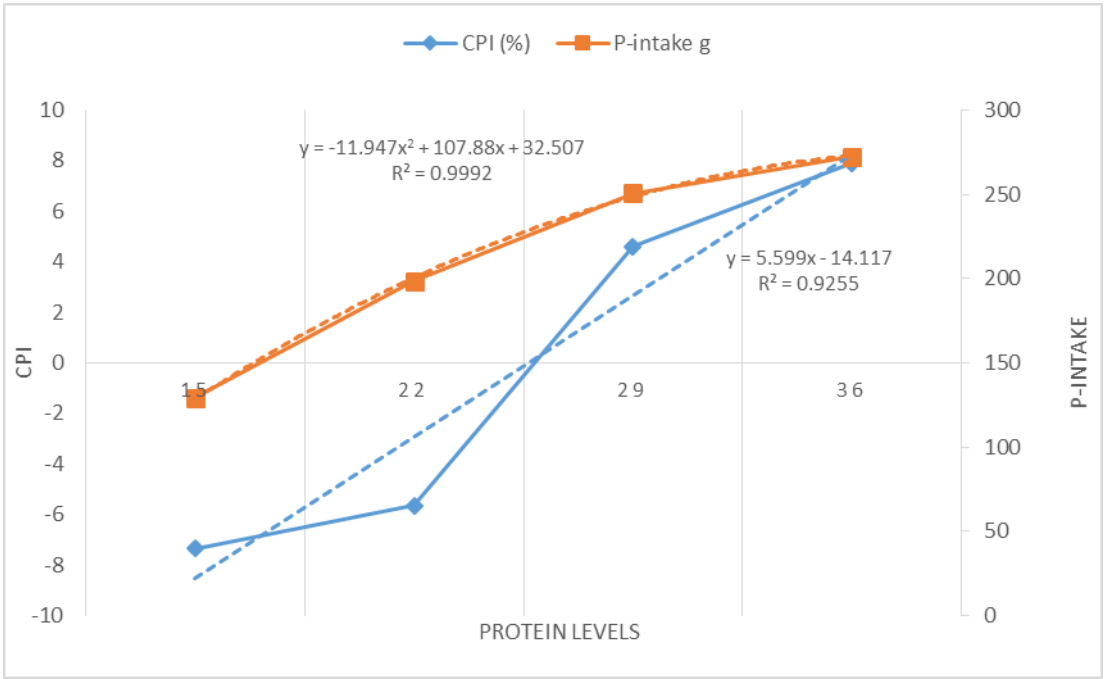


Figure 1: The relationship between CPI and protein intake.

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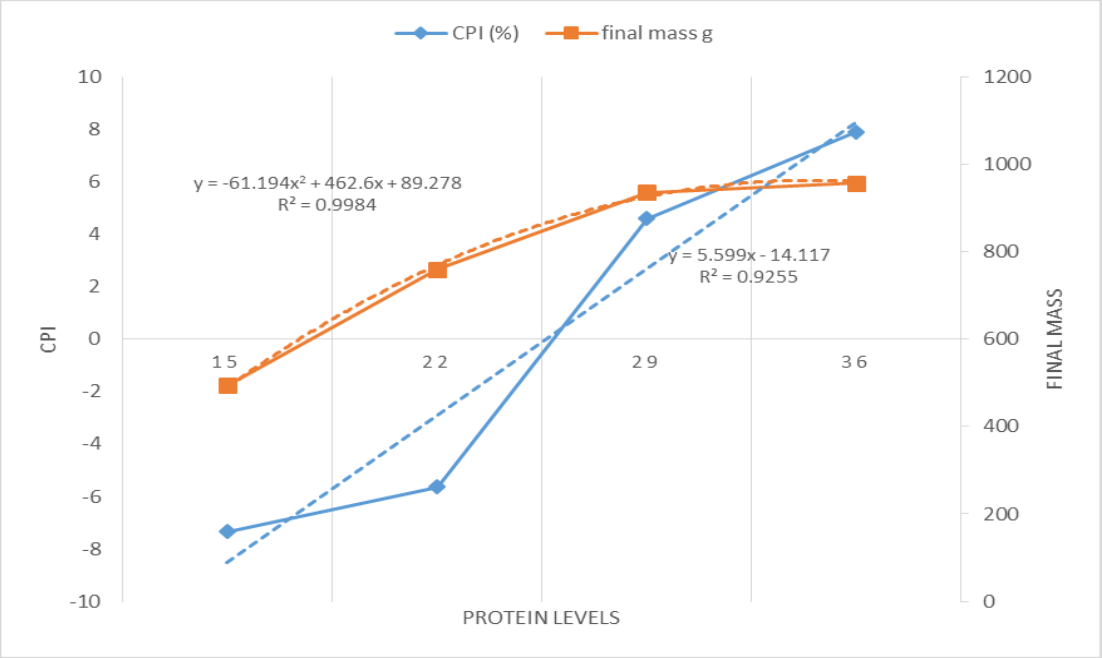


Figure 2: the relationship between CPI and final body mass.

Discussion

The structural role and expense of protein account are enough for being the first step in nutritional requirement studies; on the other hand, energy and non-protein energy supplier have an important effects on the protein requirement investigation; so a factorial design has been planned. In this study, different levels of protein and lipid did not have any significant effects on survival that has been showed by others authors including De Silva *et al*. (1991) on Red tilapia and Al-Hafedh (1999); Siessegger *et al*. (2006) and Lim *et al*. (2009) on Nile tilapia.

Some growth indices (WG and SGR) improved by protein accretion that is certified by De Silva *et al*. (1991), Al-Hafedh (1999), Siessegger *et al*. (2006) and Ng and Hanim (2007) on Nile tilapia *O. niloticus*. It has been proved that increasing in protein causes higher growth rate until a special level and then it reduces or fixes in higher protein levels (Jauncey, 1982; De Silva and Pereira, 1985; Moore *et al*., 1988; De Silva *et al*., 1989; Shiau and Huang, 1989; Ng *et al*., 2001; Abdel-Tawab *et al*., 2010) in this study, too. Best growth performance has been found in the lowest lipid level (5 %) and there was not any significant difference between 29 and 36 % crude protein (p>0.01); but it was reduced after 29% protein level especially in 13% crude lipid. According to these results and from an economic point of view, based on growth indices 29 % crude protein is sufficient for convincing growth rate. However, the protein requirements of Nile tilapia has been reported by different authors vary between 25–40 % crude protein (Cruz and Laudencia, 1977; Davis and Stichney, 1978 (fresh water); Wang *et al*., 1985b; Shiau *et al*., 1987 (fresh water); Siddiqui *et al*., 1988 (fresh water); De Silva *et al*., 1989; Shiau and Huang, 1989 (sea water); Twibell and Brown, 1998 (fresh water);

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Al-Hafedh, 1999 (salinity: 1.5 ppt); Ahmad *et al*., 2004 (fresh water); El-Saidy and Gaber, 2005 (fresh water); Siessegger *et al*., 2006 (fresh water[1])). This wide range resulted in different experimental conditions such as species, size, age, density, protein quality, environmental condition, temperature, salinity and other unknown factors (Jauncey and Ross, 1982; Wilson, 1989; El-Sayed and Teshima, 1992; Al-Hafedh, 1999; Ahmad *et al*., 2004), iso-energetic diets because of the importance of protein-energy ratio (De Silva and Pereira, 1985; Wang *et al*., 1985a,b; Mazid *et al*., 1979; Siddiqui *et al*., 1988) and difference feed consumption (Kaushik *et al*., 1995).

In this study, growth indices were reduced by lipid increasing in all protein levels; therefore, tilapia could not grow more by extra energy and protein-sparing effect did not happen, actually. It was explained by Hanley (1991), Liebert *et al*. (2006) and Lim *et al*. (2009). It may be happened for high lipid because less energy was deposited in the form of protein and proportionally more was deposited as lipid reserves and weight increase of lipid is eight times less than protein and is not enough to effect on growth and final biomass significantly (Bromley, 1980; Hanley, 1991). On the other hand, high lipid can be a growth inhibitor and in optimum protein level, excess lipid cannot be made growth (Ringrose, 1971; Berge and Storebakken, 1991; De Silva *et al*., 1991).

According to the growth indices, 5% crude lipid is the best for all male Nile tilapia in brackish water. It was expressed 5-9% by Hanley (1991), and convinces level 12% and minimum level 5% by Chou and Shiau (1996). In 5% lipid level, 33.9% crude protein made maximum specific growth rate based on polynomial function between protein levels and SGR (Maynard and Loosli, 1969; Colvin and Brand, 1977; Clifford and Brick, 1978; De Silva *et al*., 1989; Al-Hafedh, 1999). According to the mathematical models (Broken line and Quadratic curve), minimum protein requirement of hybrid tilapia *O. niloticus*×*O. aureus* with an initial body weight 21 g was 28 percentage (Twibell and Brown, 1998).

There was a significant interaction within different levels of protein and lipid on FCR (p<0.01). Winfree and Stickney (1981) confirmed this on blue tilapia *Tilapia aurea*. FCR was reduced by protein increasing, significantly (p<0.01). Therefore, best one happened in the diet with 36% CP; but, it did not have any significant difference with 29% CP (p>0.01). FCR improvement with protein accretion has been reported on Nile tilapia (De Silva *et al*., 1991; Al-Hafedh, 1999; Ahmad *et al*., 2004; Ng and Hanim, 2007). In fact, in a high level of protein, FCR causes better growth rate in fish (Gunasekera *et al*., 1995). At the minimum protein level, FCR increased significantly by lipid accretion (p<0.01). This is across what was reported by Wille *et al*. (2002) and Sweilum *et al*. (2005); presumably,

[1] Fresh water was considered for the articles that did not mention to the salinity.

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because of the negative effect of higher lipid levels. However, there was not any significant difference in FCR in sufficient protein level (p>0.01) that was confirmed by Hanley (1991) and Gao *et al*. (2011).

Total feed intake increased up to 29% crude protein. The same results were reported on Nile and red tilapia (Abdel-Tawwab *et al*., 2010; De Silva *et al*., 1991), respectively. Stomach capacity was an inhibiting factor in 15% CP for supplying energy and protein requirements like what reported for fry of blue tilapia *Tilapia aurea*, Nile tilapia and hybrid tilapia *O. niloticus*×*O. aureus* (Winfree and Sickney, 1981; Day *et al*., 2008; Gao *et al*., 2011), respectively. Total feed intake decreased by lipid increasing except 15% CP. More energy in a diet made less feed intake because the fish get their energy requirement by a smaller amount of feed, completely. If fish use diets with high energy, their growth will be lower as a consequence of lower feed consumption and lower protein intake (Ellis and Reigh, 1991; Kaushik and Medale, 1994; Wang *et al*., 2005; Gao *et al*., 2011). Protein intake increased by protein accretion, significantly too. However, lipid increasing affected conversely, because of the higher energy of diet and less feed intake.

The pattern of increasing and decreasing of energy intake corresponded to the growth of tilapia, cod too (Helland *et al*., 2008). Although different treatments had the same energy intake, those used diets with low level of protein could not be able to grow adequately. When turbot fed near satiation, like this experiment, energy intake resembled each other, and total feed intake seemed to depend on energy of diets to protein levels (Bromley, 1980). Some authors showed fish consume diets until supply their requirement when they feed near satiation (Lee and Putnam, 1973; Page and Andrews, 1973; Ellis and Reigh, 1991; Kaushik and Medale, 1994). Tilapia regulated their total feed intake based on energy intake, because there was the same energy intake in all treatments and the difference of energy of diets did not affect. Rainbow trout regulate feed intake according to available energy, too (Kaushik *et al*., 1981; Kaushik and Luquet, 1984).

Protein efficiency ratio (PER) and protein conversion efficiency (PCE) show quality and quantity of protein and amino acids balances (Ahmad *et al*., 2004). At all lipid levels, PER increased by protein accretion up to 29% crude protein, but not to be significant among 22, 29 and 36% crude protein; Just like what found on hybrid tilapia *O. niloticus*×*O. aureus* (21 g) between 24 – 34 % crude protein by Twibell and Brown, 1998. A bit reduction found in PER by lipid increasing in all protein levels, except 15% CP, was not significant that is confirmed by Chatzifotis *et al*. (2010) and Gao *et al*. (2011) on meagre *Argyrosomus regius* and hybrid tilapia *O. niloticus*×*O. aureus*, respectively. Protein conversion efficiency increased by protein accretion up to 29% crude protein and then reduced, but not significantly; it has reported by others

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that the best protein and energy utilization in fingerling Nile tilapia was in 30% digestible protein (Kaushik *et al*., 1995). According to the polynomial curve between protein levels and PCE in 5% crude lipid, maximum PCE happened in 35.3% crude protein; in result, it proved protein de-amination and use of protein as an energy source after this level (De Silva and Pereira, 1985; Kim and Lee, 2009; Abdel-Tawwab *et al*., 2010). In this study, PCE increasing by protein enhancement reverse on what have found by some authors (Ahmad *et al*., 2004; Sweilum *et al*., 2005; Kim and Lee, 2009; Singh *et al*., 2009). Probably, it reduces by protein increasing when protein maintenance requirement is supplied. So, in 15 and 22% CP, because of low protein level, it increased up to 29% CP and then reduced; it confirmed by De Silva *et al*. (1991).

Protein level could be reduced without any negative effects on growth if energy supplying has been done by non-protein resources. It is proved for brown trout *Salmo trutta*, channel catfish *Ictalurus punctatus* and yellow tail *Seriola quinqueradiata* (Winfree and Stickney, 1981). The protein sparing effect has been reported on tilapia at 12 and 18 % CL (De Silva *et al*., 1991; Chou and Shiau, 1996), respectively. In this study, protein efficiency increased up to 9% CL and then reduced but not significantly; so, protein-sparing effect appeared, unclearly. In addition, tilapia cannot be able to use lipid as an energy source if the protein level of diet will not be limited (Hanley, 1991; Peres and Oliva-Teles, 1999). This was reported on Cod, too (Helland *et al*., 2008).

When there is not enough maintenance protein requirements, fish use amino acids of their body to compensate, so it effects on their growth and carcass protein percentage. Thus, protein intake at the point that CPI (carcass protein increasing) is zero could may be maintain protein quality (carcass protein percentage). In this study at 25.6%, CP fish could prevent losing their carcass protein percentage (Figs. 1 and 2). At this point, their protein requirement was 265 mg protein per g fish.

Unlike protein, lipid increasing did not effect on carcass protein, significantly. Adversely, Sweilum *et al*. (2005) and Hanley (1991) reported that the carcass protein of Nile tilapia decreased by energy enhancement of the diets. Carcass protein increased by protein increasing that is confirmed by others (Sweilum *et al*., 2005; Liebert *et al*., 2006; Siessegger *et al*., 2006). Protein enhancement significantly decreased carcass lipids, especially in high levels of protein. Some authors explained the same results (Sweilum *et al*., 2005; Siessegger *et al*., 2006; Kim and Lee, 2009). Protein accretion caused protein enhancement and lipid reduction in the carcass; inverse relationship between protein and lipid of the carcass was made from protein increasing of diets on Channel catfish and Nile tilapia (Garling and Wilson, 1976; El-Saidy and Gaber, 2002, 2005), respectively. In conclusion, growth, feed and protein performances improved by protein increasing. It is

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recommended to use 29% CP, and 5% CL in diets for tilapia rearing.

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