The effect of dietary lipid level on the growth of kutum fry (*Rutilus frisii kutum*)

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Abstract

The study was conducted with a completely randomized design consisting of four treatments in triplicates. Experimental diets were formulated to contain four different lipid levels (8, 10, 12 and 14%) at constant protein level (46.8%) and different gross energy of 4314, 4417, 4519 and 4622kcal/kg, respectively. Kutum fry (average weight, 203±4mg) were randomly assigned and distributed at a density of 2 fish/l into twelve 80 liter fiberglass tanks equipped with a tap-freshwater system and water temperature of 22-24°C. Fish were fed the experimental diets thrice a day at 10% of body weight for 81 days. Statistical analysis indicated that fish fed the lowest lipid level of 8% showed significant differences ($P<0.05$) with other treatments. Fish growth, survival, protein and energy retention showed a marked decrease with an increase of dietary lipid level. Crude lipid of carcass composition increased, but ash and moisture decreased with an increase of lipid levels.

Keywords: *Rutilus frisii kutum*, Dietary lipid, Nutritional performance, Feed conversion ratio

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Introduction

Lipids are important components of a diet as both energy and essential fatty acids sources required for basic functions, includeing maintenance, growth, and as carrier for oil-soluble vitamins. It is well known that lipids play an important role in the cell structure, membrane of tissues and some are rich in ω-3 polyunsaturated fatty acids (PUFA), which are essential for fish (Regost *et al.*, 2001).

Protein utilization for fish growth can be improved by partially replacing protein with lipid or carbohydrate in the diet. Improper dietary protein and energy levels and their ratios will lead to lower fish performance. Thus, it is important to determine the proper levels of carbohydrates and lipids as well as protein content in diets (Yang *et al.*, 2002). The current trend in fish feed production is to increase the lipid content in diets to spare protein, improve feed conversion and decrease the amount of waste produced by the fish.

Kutum is a native fish of the southern parts of the Caspian Sea and it can reach a size of 6kg (Azari *et al.*, 1990). The excellent quality and taste of the meat make this species one of the most popular food fish along the Iranian coasts of the Caspian Sea. Due to over-exploitation of the stocks, wild population had decreased in the last decades (Holcik, 1995). In recent years, Iranian aquaculture production of kutum fingerlings is estimated to be above 100 millions, which are released in the southern rivers flowing into the Caspian Sea to restock wild population. Despite the rapid development in the culture of kutum larvae and a steady improvement in food formulation, the nutritional requirements of this potentially important species have not been evaluated. The present study is the first of its kind to provide a basic information required for the formulation of lipid level for kutum.

Materials and methods

The kutum fry of 203±4mg average weight from Shahid Ansari Hatchery Complex were acclimated to the experimental condition and initially fed with a commercial diet containing 38% crude protein for 14 days. They were randomly distributed into 120 liter round fiberglass tanks, filled with 80 liter de-chlorinated tap water, at a density of 160 fish per tank (2 fish/l). Fish wet weight and total length were measured at day 0 and then at 10 day intervals for 81 days.

The completely randomized design of this experiment consisted of four treatments in triplicates. The fish were not fed for 24 hours prior to the feeding trials. During the feeding trial, fish were hand fed with the experimental diets with a particle diameter of 2.2% of the total mean length of the fish (Uys & Hecht, 1985).

The tanks were continuously aerated through aeration stones. Feeding was performed three times a day at 09:00, 13:00

and 17:00h at a daily ration of 10% of biomass. Water temperature, pH, un-ionized ammonia and dissolved oxygen were monitored twice daily at 09:00 and 16:00h and set to range between 22-24°C, 7.0-7.5, 0.0012-0.0036mg/l and 8-10mg/l, respectively.

Four isonitrogenous diets with 46.8% crude protein (CP) were formulated (Table 1) based on Lindo Program (1998) to contain 8, 10, 12 and 14% crude lipid (CL). The gross energy levels of the diets were 4314, 4417, 4519, 4622kcal/kg, respectively. Fishmeal (kilka) was the major protein source in all diets. Corn and wheat flours were used as carbohydrate sources.

Table 1: Ingredients and proximate composition (% as fed basis)

Ingredients (%)	Experimental diets			
	8%	10%	12%	14%
Fish meal	52	52	52	52
Soybean meal	24	24	24	24
Wheat flour	5	5	4	4
Corn flour	7	5	4	2
Fish oil	8	10	12	14
Vitamin premix[1]	2	2	2	2
Mineral premix[2]	2	2	2	2
Proximate composition				
Dry matter (DM)	98.4±0.1	97.1±0.2	96.7±0.1	97.8±0.1
Ash (%)	7.63±0.3	8.1±0.1	7.9±0.2	7.72±0.1
Crude protein (%)	46.8	46.8	46.8	46.8
Crude lipid (%)	8.32	10.81	12.83	14.28
Gross energy (Kcal)	4314	4417	4519	4622

Vitamin premix[1] (g/kg): Vitamin A acetate 1000,000 IU; Vitamin D_3 400,000 IU; Vitamin E 20,000 IU; Thiamin HCI 0.5; Riboflavin 3; Pyroxidine HCl 1; D-calcium pantothenate 5; Nicotinic acid 5; Bioton 0.05; Folic acid 0.18; B_{12} 0.002; Choline chloride 100; Inositol 5; Menadione 2.

Mineral premix[2] (g/100g): Ferrous sulfate 4; Magnesium sulfate heptahydrate 28; Potassium iodide 0.067; Cobalt chloride 0.004; Cupric sulfate monohydrate 0.650; Sodium selenite 0.010; Zinc sulfate heptahydrate 13.

At the end of the growth trial, the fish were individually weighed, their viscera and liver removed to determine hepato-somatic index (HIS = liver weight × 100/fish weight) and viscera-somatic index (VSI = visceral fat weight × 100/fish weight).

The fish were homogenized as a group and frozen at -20°C for the subsequent analysis according to procedures of AOAC (1998).

Growth parameters and nutrient utilization were calculated according to the following formulae (Bromley, 1980; Murai *et al.*, 1985):

Weight Gain (WG) = 100 × ((final weight - initial weight) / initial weight))

Specific Growth Ratio (SGR) = 100 × ((L_n final weight - L_n initial weight) / days)

Feed Conversion Ratio (FCR) = dry weight feed offered / (final weight - initial weight)

Protein Efficiency Ratio (PER) = (final weight-initial weight) / protein offered

Protein Conversion Efficiency (PCE) = (protein deposited in fish/protein intake) × 100

Energy Conversion Efficiency (ECE) = (energy deposited in fish / energy intake) × 100

Condition Factor (CF) = (weight / length ^{3}) × 100

Survival = (final number fish – initial number fish) × 100

All data were statistically analyzed by one-way analysis of variance (ANOVA) to test the effects of dietary lipid level on growth performance and proximate composition of fish body. Multiple range testing, Duncan was used to compare mean differences (Steel & Torrie, 1960). Growth responses in the terms of average weight gain to dietary lipid estimated by the regression analysis (polynomial) were also applied where appropriate (Snedector & Cochran, 1971). The statistical tests were performed using a SAS program (SAS Institute, 1985).

Results

After the 11 week feeding trial, the mortality of the fish was low and did not exceed 5% in all treatments (Table 2). Growth performance was highest at 8% dietary lipid level (gross energy 4314kcal/kg) and significantly ($P<0.01$) decreased as the lipid level increased (Table 2); the highest specific growth rate (SGR) of 2.78 (%day $^{-1}$) was also recorded in fish fed 8% lipid level. Polynomial regression analysis between lipid levels and weight gain of fish indicated higher weight gain at 8% dietary lipid level (Fig. 1).

Table 2: Growth performance of kutum fry fed on iso-nitrogenous diets containing different lipid levels over 11 weeks at 22-24°C

Growth parameters	Lipid level (%)			
	8	10	12	14
Final weight (g)	1926±36.66[a]	1715±15.32[b]	1701±15.32[b]	1577±52.18[b]
Final length (cm)	56±0.942[a]	56±0.471[b]	54±0.816[bc]	52±1.24[bd]
Total food intake (mg)	3412±25.92[a]	3346±64.50[a]	3293±94.94[a]	3245±109.13[a]
WG (%)	852±22.44[a]	760±4.89[b]	737±8.64[b]	671±24.14[c]
SGR (%/day)	2.78±0.029[a]	2.62±0.026[b]	2.62±0.008[b]	2.51±0.041[b]
PER	1.07±0.040[a]	0.987±0.026[b]	0.971±0.018[b]	0.904±0.013[b]
PCE (%)	42.5±0.816[a]	40±0.000[b]	39±0.816[bc]	37±0.816[cd]
ECE (%)	38.6±1.247[a]	37.6±0.471[a]	36.6±0.471[ab]	35±0.816[bc]
FCR	2.05±0.027[c]	2.16±0.059[bc]	2.20±0.040[b]	2.36±0.035[a]
Survival (%)	97.5±2.041[a]	95±0.000[a]	96.6±1.178[a]	95±0.000[a]

Values are means±standard deviations (n=3). Values in the same row with different superscripts are significantly different (P< 0.05).

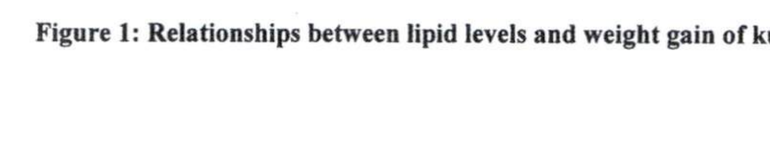

Figure 1: Relationships between lipid levels and weight gain of kutum fry

Protein efficiency ratio (PER) decreased with increasing dietary lipid level and was significantly higher (P<0.01) at 8% lipid level (Table 2). Similar observations were made for conversion efficiencies for protein (PCE) and energy (ECE). Feed conversion ratio (FCR) increased with an increase in dietary lipid level.

The highest condition factor (CF) value of 1.121 was obtained at 12% lipid level, which was not significantly different from 14% level, but significantly differed (P<0.01) from 8% and 10% lipid levels (Table 3). The highest hepato-somatic index (HSI) of 1.90 was observed at 8% lipid treatment, which was not significantly different from other treatments. Visceral-somatic index (VSI) increased with an increase in dietary lipid levels. The highest VSI was recorded at 12% dietary lipid levels, which was significantly different (P<0.01) from 8% and 10% levels.

The proximate compositions of whole body carcass presented in Table 3. Fish moisture content decreased (P<0.05) with an increase in dietary lipid level from 8-10% to 12-14%. Fish crude protein was not affected by dietary lipid levels. Fish crude lipid content significantly increased (P<0.05) in direct proportion to the dietary lipid levels. Fish ash content decreased significantly (P<0.05) with an increase in dietary lipid level (Table 3).

Table 3: Proximate analysis of the whole body and morphological measurements of kutum fry fed diets containing different lipid levels (% of wet weight basis)

	lipid level (%)			
	8	**10**	**12**	**14**
Morphological measurements				
VSI (%)	8.466 ± 0.077^{bc}	9.316 ± 0.085^{b}	12.406 ± 0.563^{a}	11.721 ± 0.61^{a}
HIS (%)	1.90 ± 0.153^{a}	1.73 ± 0.075^{a}	1.77 ± 0.072^{a}	1.65 ± 0.078^{a}
CF	1.058 ± 0.008^{bc}	1.049 ± 0.006^{b}	1.121 ± 0.019^{a}	1.120 ± 0.016^{a}
Organic matters				
Moisture (%)	67.83 ± 0.141^{a}	67.54 ± 0.081^{a}	66.46 ± 0.083^{b}	66.31 ± 0.021^{b}
Protein (%)	18.23 ± 0.08^{a}	18.34 ± 0.49^{a}	18.16 ± 0.22^{a}	17.92 ± 0.11^{a}
Lipid (%)	7.83 ± 0.07^{a}	8.55 ± 0.10^{b}	9.67 ± 0.08^{c}	10.16 ± 0.14^{d}
Ash (%)	4.38 ± 0.113^{a}	3.13 ± 0.149^{b}	2.86 ± 0.049^{bc}	2.79 ± 0.055^{d}

Values are means±SD (n=3). Values in the same row with different superscripts are significantly different (P< 0.01).

Discussion

Lipids are the most important source of energy for fish as they are highly digestible. They are well metabolized and, consequently, spare protein to produce a maximum growth (Watanabe, 1982). However, too much lipid in the diet may decrease the weight gain (Murai *et al.*, 1985; Williams *et al.*, 1985; Weatherup *et al.*, 1997; Silverstein *et al.*, 2000) while a lower lipid level has also been found to bring about a higher feed performance in the red drum, *Sciaenops ocellatuss* (Julio *et al.*, 1992).

In general, 4–25% lipid in fish diets gives optimal growth rates without producing an excessively fatty carcass (Ding, 1991). Optimal lipid level has been suggested to be below 12% in the practical diets of many cyprinids (Kaushik, 1995). For grass carp, less than 4% lipid was recommended (Ding, 1991) which it is comparatively lower than that for most other teleosts.

In the present study, all indicators of growth performance and nutrient retention such as WG, SGR, PER, PCE, ECE and FCR improved as the dietary lipid levels decreased, which was a clear implication of reduced protein retention as lipid level increased from 8% to 14% (Table 2). The weight gain of fish is usually a reliable indicator of the nutritional adequacy of the diet (Cho & Watanabe, 1985). The diet containing 8% lipid level (4314kcal/kg gross energy) yielded the highest weight gain performance in kutum fry (Fig. 1). An increase in dietary lipid content at any given crude protein level was found to reduce growth and feed efficiency or protein retention in juvenile carp (Murai *et al.*, 1985), which corresponds with our findings on kutum fry. Similar findings have also been reported in other fish species (Regost *et al.*, 2001; Hebb *et al.*, 2003; Lin & Shiau, 2003).

The FCR increased with increase in lipid levels from 8% to 14% (Table 2), which corresponds with earlier reports on Nile tilapia, *Oreochromis niloticus* (Siddiqui *et al.*, 1988), red drum, *Sciaenops ocellatus* (Julio *et al.*, 1992), golden shiners, *Notemigonus crysoleucas*, and goldfish, *Carassius auratus* (Lochmann *et al.*, 1994), juvenile *Zacco barbata* (Shyong *et al.*, 1998), and spotted sand bass, *Paralabrax maculatofasciatus* (Grayeb-Del Alamo *et al.*, 1998). In the present study, the FCR seems to be high because the total food supply has been taken into account, irrespective of wasted feed.

Protein efficiency ratio (PER) is an important measure of the protein utilization efficiency. PER value decreased with an increase in dietary lipid levels (Table 2), which corresponds with earlier reports (Ballestrazzi *et al.*, 1994; Robaina *et al.*, 1997; Shyong *et al.*, 1998).

A high condition factor (CF) is usually considered to reflect the storage of energy reserves (Birkett, 1972). CF in *R. frisii kutum* increased with an increase in dietary lipid level (Table 3), which was relatively convergent with results on other species such as turbot, *Scophthalmus maximus* (Bromley, 1980), yellowtail, *Seriola dumerilii* (Jover *et al.,* 1999), rockfish, *Sebastes schlegeli* (Lee *et al.*, 2002), and silver perch, *Bidyanus bidyanus* (Yang *et al.*, 2002).

Energy conversion efficiency (ECE) decreased with an increase in dietary lipid level (Table 2). ECE was significantly ($P<0.05$) influenced by the composition of the diet. When protein and energy retention are increased, higher nutrient utilization and less wastes, especially nitrogen excretion, is expected (Viola & Lahav, 1991).

Protein conversion efficiency (PCE) ranged from 37% to 42.5% in this work, with the upper limit recorded at 8% lipid level. Although the normal level of energy retention is around 30%, higher values ranging from 40% to 60% have been described (Garling & Wilson, 1976). Cho and Watanabe (1985) observed that the highest dietary lipid did not promote the highest lipid retention. In addition, fish tend to adjust their feed intake in accordance with their energy requirements (Smith, 1989).

Lipids seem to be mainly deposited in the viscera. This was confirmed by the increase in viscera-somatic index (VSI), but hepato-somatic index (HSI) decreased as dietary lipid level increased. In this trial, lipid was essentially stored in the viscera and relatively low in the liver. In the lipogenic process, high fat diets depress the activities of several lipogenic enzymes such as fatty acid synthetase (FAS), malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G6PD) in marine fish like sea bass (Bautista *et al.,* 1988; Dias *et al.,* 1998). It is known that even a slight increase in dietary fat reduces the activity of these enzymes (Kelley *et al.*, 1987). Shimeno *et al.* (1981) found that increase in dietary carbohydrate and fat caused a reduction in the activities of enzymes in the hepato-pancreas.

The whole carcass composition analysis showed that as dietary lipid increased, the moisture and ash contents decreased. Crude lipid content increased with increase in dietary lipid, but protein content remained constant (Table 3). This agreed with earlier works on various fish species (Page & Andrews, 1973; Peres & Oliva-Teles, 1999; Regost *et al.*, 2001).

From the overall results of this work, it can be concluded that a dietary lipid level of 8% and 4314kcal/kg brought about a better growth performance in kutum, *R. frisii kutum*, fry than the higher tested lipid levels.

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