Dietary Lipid Requirement for the Kutum Fingerlings, *Rutilus frisii kutum* (Kamenskii 1901)

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Abstract: Growth trials were conducted to investigate the effects of dietary lipid levels on growth, feed utilization, condition factors, survival rate and carcass composition and to evaluate the lipid requirements of kutum fingerlings, *Rutilus frisii kutum*. Five iso-nitrogenous experimental diets were formulated to contain graded levels of lipid (50, 80, 120, 160 and 190 g kg^{-1}). After 2 weeks of the conditioning period, triplicate groups of 80 kutum (initial average weight of 0.50 ± 0.07 g) were stocked in 250-l tanks and fed to apparent satiation thrice a day for 8 weeks. After feeding trials, the results showed that growth performance, feed intake, specific growth rate, protein and energy efficiency ratio, energy retention ratio and whole-body composition were significantly affected by dietary treatment. The survival rates were generally high, from 95% to 99.58% over the eight-week trial. Growth performance generally improved with increasing dietary lipid from 50 to 120 g kg^{-1} lipid diets and slightly decreased thereafter with further increase in dietary lipid. Significantly higher growth (P<0.05) was achieved in kutum fed the diet containing 120 g kg^{-1} lipid. Food conversion ratio was negatively correlated with the dietary lipid ($R^2 = 0.897$). When the dietary lipid level was higher than 120 g kg^{-1} lipid diets, this nutrient did not significantly affect food conversion ratio. Kutum fed the diet with 190 g kg^{-1} lipid had the highest protein retention efficiency and energy retention efficiency among the dietary treatments. Protein retention efficiency and energy retention efficiency, generally increased with increasing dietary lipid level but lipid retention efficiency were inversely correlated with dietary lipid concentration (P<0.05). The carcass composition of kutum was affected by dietary treatments except for ash and protein contents. The carcass lipid of fish in this study was obviously increased corresponding to dietary lipid levels. Based on the results of this study, the maximum growth of kutum fingerlings was observed when fed the diet containing 120 g kg^{-1} lipid diets and the protein/energy ratio was approximately 21.68 g/MJ. The optimal dietary lipid level, estimated by polynomial regression analysis, for maximum growth of kutum fingerlings was 13.55%.

Key words: Kutum *Rutilus frisii kutum* · Lipid requirement · Growth · Food intake · Body composition

INTRODUCTION

Dietary lipid is an important nutrient element for fish nutrition, which supplies a source of energy and essential fatty acids and is also transporters of fat-soluble vitamins [1]. Extreme lipid or carbohydrate (non-protein) energy sources in diet may have adverse influences on growth, reduce feed intake and inhibit the utilization of other nutrients and lead to increased fat deposition [2, 3]. Use of appropriate dietary lipid level is prevented to employ the dietary protein for energy generation, resulting in higher specific dynamic action and will lead to a decrease in ammonia excretion and the feed costs [4, 5]. Protein sparing effect implies that inclusion of lipid and carbohydrate (non-protein) energy sources has been displayed to lower dietary protein employed for energy and elevate protein utilization for growth [6]. Dietary lipid has been shown to provide a protein sparing effect in some fishes [7-10].

The effects of dietary lipid level on growth rate, flesh quality, immune response and disease resistance and reproduction performance have been investigated in several species important in aquaculture [11-14]. El-Sayed and Garling [6] reported that, on *Tilapia zilzii* increasing

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dietary lipid level up to 15% caused a significant improvement in protein utilization and growth performance. Hardy [15] indicated that Atlantic salmon were able to use diets containing upwards of 30% lipid and were able to utilize this energy source to significantly improve feed and protein utilization efficiencies and to reduce nitrogen excretion.

Fishing in Caspian Sea concentrates mainly on two fish species: Kutum and Common carp, both being appropriate growth rate. But kutum, *Rutilus frisii kutum* (Kamenskii 1901), is a very popular food fish because of its good meat quality and good consumer acceptance, especially in north of Iran. The fish resources in Caspian Sea were greatly damaged, because of destruction of natural spawning areas, the natural reproduction of kutum has been limited and its artificial reproduction is successfully done in hatcheries [16]. Nowadays, annually to restock this valuable species, the Iranian fisheries organization (Shilat) produces and releases more than 150 million fingerlings into rivers which carry them toward the Caspian Sea (www.shilat.com). The annual landing of kutum rose from 563 mt in 1982 to 16118 mt in 2006 [17].

Regarding feeding habits, kutum fry has been reported as an omnivorous species [18]. In the restocking centers, larval rearing takes place in earth ponds and kutum larvae feed on zooplankton and formulated diet. The formulated diets are used in the form of paste and contain 28-35% protein and 8-10% lipid, based on the requirements for common carp (*C. carpio* L) because nutritional requirements of this species are still unknown. In practice, kutum fed normally on zooplankton and formulated diets (co-feeding) reach a release size (0.5-1.5 g) in 70-90 day.

Although several studies have been conducted to appraise the nutritional parameters of kutum [19-21], there is no accurate information on the dietary lipid requirement of kutum. Therefore, the present study was designed to evaluate the effect of lipid levels in a practical diet on growth rate, feed utilization and body composition of kutum, *Rutilus frisii kutum* fingerlings.

MATERIALS AND METHODS

Experimental Fishes: Kutum (*Rutilus frisii kutum*) fingerlings were obtained from Shahid Rajaee Restocking Center located in the southern coast of Caspian Sea, Sari, Iran. Before starting the experiment, the fish were acclimated to the experimental conditions for 2 weeks. During acclimation, fishes were fed with commercial carp feed (32% protein and 10% lipid) twice daily. After acclimation, kutum fingerlings with initial body weight of 0.5 ± 0.09 g were randomly distributed into 250 liter fiberglass tanks with 80 fish per tank. Each dietary treatment had 3 replications. All tanks were covered with nets throughout the experiment, to prevent fish from jumping out.

Rearing Conditions: During the trial period, freshwater was supplied at a flow rate of 1.5 l/min to each tank. Water quality parameters were checked periodically, pH was adjusted between 7.8-8.0, water temperature ranged from 19.7 to 23.2°C and dissolved oxygen was not less than 7.2 mg/l. Fish were held under natural photoperiod condition throughout the feeding trail. Fish were hand-fed with the experimental diets until apparent satiation three times daily (7 days a week) at 0700, 1200 and 1700 h for 8 weeks. Feed consumption was recorded for each tank. At the end of experiment period, fish were fasted for 24 h and total weight of replicates recorded.

Diet Preparation: Five iso-nitrogenous diets with 42% crude protein (CP) were formulated to provide five dietary lipid levels at 50, 80, 120, 160 and 190 g kg^{-1} diet (dry basis). Ingredients were obtained from a local feed manufacture (khorak dame mazandran, Sari). Gross energy content of each diet was calculated based on 23.4, 39.2 and 17.2 MJ/kg for protein, lipid and carbohydrate, respectively. Feed stuffs were homogenized through a 1.0 mm sieve, blended in the computed ratios, moistened with distilled water (25% v/w) and pelleted in a grinder. Diets were placed in a forced ventilation oven (24 h) for complete drying at 60°C. Dried pellets were sealed in plastic bags and stored frozen (-10°C) until fed. The formulation and proximate analysis of experimental diets are shown in Table (1).

Sample Fish and Chemical Analysis: At the start of experiment, 50 fish from the original population were fasted for 24 h, euthanized and stored at -20°C for initial whole body composition analysis. At the end of the experiment, 20 fish from each tank were randomly sampled and euthanized by overdose of ethylene glycol monophenyl ether and kept frozen (-20°C) for determination of final whole body composition. The analysis of diets and fish samples for crude protein, crude fat, moisture and ash followed standard Association of Official Analytical Chemists [22]. Crude protein content (N×6.25) was determined according to the Kjeldahl

Table 1: Formulation and proximate composition of diets used in the experiment

	Dietary lipid level (g kg^{-1} diet)				
Ingredient (g)	50	80	120	160	190
Fish meal[a]	540	540	540	540	540
Wheat flour	300	300	300	300	285
Fish oil[b]	0.0	7	34	60	70
Soybean oil	7.5	25.5	38.5	52.5	64
Mineral mixture[c]	20	20	20	20	20
Vitamin mixture[d]	20	20	20	20	20
Cellulose[e]	112.5	87.5	47.5	7.5	1
Total	1000	1000	1000	1000	1000
Proximate composition (g kg^{-1} diet)					
Moisture	92.3	89	96.8	105.6	104.8
Crude protein	419.1	417.2	414.1	413.2	415.4
Lipid	53.7	88.1	119.3	168.9	188.2
Ash	94.8	93.9	94.8	102.8	96.7
NFE[f]	340.1	311.8	275	209.5	194.9
Gross energy (MJ/kg)[g]	17.76	18.57	19.09	19.75	20.45
P/E ratio[h]	23.59	22.45	21.68	21.22	20.31

[a] Pars kelika Co. Mirood, Iran

[b] Herring oil

Vitamin premix contained the following vitamins (each kg $^{-1}$ diet): vitamin A, 10 000 IU;

vitamin B_6 20 mg; vitamin B_{12}, 0.04 mg; biotin, 0.2 mg; choline chloride, 1200 mg; folic acid, 10 mg; inositol, 200 mg; niacin, 200 mg; pantothenic calcium, 100mg.

[d] Contained (g kg^{-1} mix): MgSO$_4$.2H$_2$O 127.5; KCl, 50.0; NaCl, 60.0; CaHPO$_4$,.2H$_2$O, 727.8;

; Ca(IO$_3$)$_2$. 6H$_2$O, 0.295; CrCl$_3$,6H$_2$O, 0.128.

[e] Sigma, St. Louis, MO, USA..

[f] Nitrogen-free extract (NFE) = 1000-(protein + lipid+ moisture+ ash).

[g] Based on 23.4 KJ/g protein, 39.2 KJ/g lipid and 17.2 KJ/g NFE.

[h]Protein/Energy ratio (g/MJ).

method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by ether extraction using a Soxtec extraction System HT (Soxtec SystemHT6, Tecator, Sweden). Ash was determined by muffle furnace at 550°C for 24 h. Moisture was determined by oven-drying at 105°C for 24 h.

Calculations: The performance parameters including percentage weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, feed efficiency ratio, feed intake, proteins intake and survival percentage were calculated as below:

- Weight gain = $[(W_F - W_I)/W_I] \times 100$
- Specific growth rate = $[(\text{Ln } W_F - \text{Ln } W_I)/T] \times 100$
- Feed conversion ratio = [Total feed intake (g)/Total wet weight gain (g)]
- Protein efficiency ratio = [Wet weight gain (g)/Total protein intake (g)]

- Feed efficiency ratio = [Wet weight gain (g)/Dry feed fed (g)]
- Feed intake = [Total feed intake/Number of fish]
- Total protein intake = [Feed intake (g) × Percent protein in the diet]
- Survival (%) = [Final number of surviving fish/Initial number of fish] × 100
- Protein retention efficiency = [(Final total body protein-initial total body protein)/total dietary protein fed] × 100
- Energy retention efficiency = [(Final total body energy-initial total body energy)/total dietary energy fed] × 100
- Condition factor = [Fish Wt (g)/Fish length (cm)^3] × 100

Where W_F and W_I are the mean final body weight and the mean initial body weight, T is the experimental duration in days.

Statistics: The data were subjected to one-way ANOVA and correlation analysis where appropriate. Multiple comparisons among means between individual treatments were made with Duncan's multiple range test. The significance level was at P<0.05. The results are presented as means ± standard deviation. All statistical analyses were performed using SPSS Base 16 for Window (SPSS Inc. 2004).

RESULTS

The survival rates were generally high, from 95.00 % to 99.58% over the eight-week trial and it was affected by the differing dietary lipid level of the diets (P<0.05). The lowest percent survival was observed for fish fed the diet containing 50 g kg^{-1} lipid diets. Final body weight, percent weight gain, survival, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and food efficiency ratio (FER) are presented in table 2. Growth rate and feed efficiency were significantly affected by dietary lipid content of the diets. Growth performance (final body weight and SGR) generally increased with increasing dietary lipid from 50 to 120 g kg^{-1} lipid diets. The highest growth performance (P<0.05) was achieved in kutum fed the diet containing 120 g kg^{-1} lipid.

The FCR of the kutum was significantly affected by dietary lipid level (P<0.05). FCR was negatively correlated with the dietary lipid (R^2 = 0.897). The high levels of dietary lipid seemed to have significantly effect on FCR (P<0.05). When the dietary lipid level was higher than 120 g kg^{-1} lipid diets, this nutrient no showed significant effect on FCR (P>0.05).

Protein efficiency and feed efficiency were improved as increasing of dietary lipid level. FER of fish fed on dietary lipid levels 120, 160 and 190 g kg^{-1} lipid was not different (P>0.05).

Body condition index was influenced by dietary lipid level. Increasing dietary lipid elevated condition factor (CF) values. The lowest value for CF occurred at the 50 g kg^{-1} dietary lipid level (P<0.05), no significant differences were discovered in the other treatments (P>0.05).

Table (3) reveals the influence of different lipid levels in the diet on protein, energy and lipid utilization of Kutum. Feed intake (FI), protein intake (PI) and lipid intake (LI) were dependent on dietary lipid levels (P<0.05). FI and PI decreased significantly (P<0.05) with increasing dietary lipid level and lipid intake tended to decrease with the decline of dietary lipid level. Kutum fed the diet with 190 g kg^{-1} lipid had the highest PRE and ERE among

Table 2: Growth response and feed efficiency of kutum fed the diets containing different lipid levels for 8 weeks.

| Treatment | Dietary lipid levels | | | | |
	50	80	120	160	190
Initial weight (g)					
Weight gain (g)	1.26 ± 0.07^{ab}	1.16 ± 0.11^{a}	1.38 ± 0.07^{b}	1.22 ± 0.09^{ab}	1.67 ± 0.12^{d}
Weight gain (%)	158.3 ± 15.6^{a}	231.5 ± 22^{b}	276.0 ± 14^{c}	247.7 ± 19^{bc}	238.7 ± 24^{bc}
FCR	3.50 ± 0.22^{c}	2.33 ± 0.20^{b}	1.85 ± 0.10^{a}	1.85 ± 0.13^{a}	1.77 ± 0.11^{a}
SGR	2.34 ± 0.14^{ab}	2.30 ± 0.12^{a}	2.54 ± 0.07^{b}	2.39 ± 0.11^{ab}	2.39 ± 0.11^{ab}
PER	0.77 ± 0.07^{a}	1.02 ± 0.09^{b}	1.28 ± 0.07^{c}	1.29 ± 0.09^{c}	1.17 ± 0.12^{bc}
FER	0.28 ± 0.02^{a}	0.42 ± 0.04^{b}	0.54 ± 0.02^{c}	0.54 ± 0.03^{c}	0.56 ± 0.03^{c}
CF	0.80 ± 0.01^{a}	0.94 ± 0.02^{b}	0.96 ± 0.07^{b}	0.97 ± 0.00^{b}	0.98 ± 0.01^{b}
Survival (%)	96.6 ± 1.9^{ab}	99.5 ± 0.7^{c}	95.0 ± 1.2^{a}	97.9 ± 1.4^{bc}	96.2 ± 1.2^{ab}

Mean values and standard deviation (M±SD) are presented for each parameter (Three groups /treatment). Means in the same line with different letters are significantly different (P<0.05).

FCR; food conversion ratio; SGR, specific growth rate; FER, feed efficiency ratio; PER, protein efficiency ratio; CF, condition factor.

Table 3: Protein, energy and lipid utilization of Kutum fed the diets containing different lipid levels. Mean values and standard deviation (±SD) are presented for each parameter (Three groups per treatment). Means in the same row with different letters are significantly different (P<0.05).

Treatment (Lipid level)	50	80	120	160	190
FI	2.70 ± 0.10^{d}	2.71 ± 0.01^{d}	2.55 ± 0.01^{c}	2.25 ± 0.04^{b}	2.07 ± 0.08^{a}
Protein PI	1.135 ± 0.449^{d}	1.138 ± 0.004^{d}	1.071 ± 0.004^{c}	0.945 ± 0.019^{b}	0.872 ± 0.036^{a}
PRE	22.46 ± 2.29^{a}	25.70 ± 2.06^{a}	27.95 ± 5.61^{ab}	27.13 ± 5.82^{a}	36.33 ± 6.41^{b}
Energy EI	48.01 ± 1.89^{c}	55.42 ± 0.21^{e}	50.74 ± 0.19^{d}	42.97 ± 0.87^{b}	38.56 ± 1.62^{a}
ERE	20.42 ± 1.55^{a}	28.69 ± 2.35^{b}	39.24 ± 2.86^{c}	47.35 ± 4.42^{d}	54.28 ± 6.87^{d}
Lipid LI	0.143 ± 0.005^{a}	0.238 ± 0.000^{b}	0.303 ± 0.001^{c}	0.378 ± 0.007^{d}	0.390 ± 0.016^{d}
LRE	67.12 ± 2.27^{d}	44.86 ± 2.82^{c}	33.84 ± 3.09^{b}	29.65 ± 1.82^{ab}	28.94 ± 14.93^{a}

FI, feed intake; PI, protein intake; PRE, protein retention efficiency; EI, energy intake, ERE, energy retention efficiency; LI, lipid intake, LRE, lipid retention efficiency.

Table 4: Carcass composition of kutum fed the diets containing different lipid levels.

Treatment	Protein	Lipid	Ash	Moisture	Energy
Initial values	12.3 ± 0.52	8.4 ± 0.11	2.8 ± 0.10	76.2 ± 0.1	642.0 ± 15.4
Whole body					
50	14.9 ± 0.32[a]	9.9 ± 0.05[a]	2.54 ± 0.11[a]	74.4 ± 0.35[c]	740.1 ± 8.5[a]
80	15.3 ± 0.23[a]	10.9 ± 0.19[b]	2.52 ± 0.24[a]	73.0 ± 0.31[b]	786.3 ± 12.2[b]
120	15.3 ± 0.59[a]	12.0 ± 0.46[c]	2.50 ± 0.16[a]	72.1 ± 0.72[b]	833.1 ± 13.1[c]
160	14.9 ± 0.49[a]	12.8 ± 0.39[d]	2.48 ± 0.24[a]	72.4 ± 0.60[b]	852.0 ± 17.4[cd]
190	15.5 ± 0.41[a]	12.9 ± 0.25[d]	2.40 ± 0.14[a]	69.9 ± 0.86[a]	871.7 ± 19.2[d]

Initial body composition: bodies of 50 fish (Means ± SD) were used for statistical analysis. Final body composition: values are means of three groups per treatment. Values in the same column with different superscripts are significantly different (P<0.05).

the dietary treatments. However, the lowest values for both parameters were recorded in fish fed the diet with 50 g kg^{-1} lipid. PRE and ERE, generally increased with increasing dietary lipid level but lipid retention efficiency (LRE) were inversely correlated with dietary lipid concentration (P<0.05).

At the end of the experiment, moisture, lipid and energy contents in body composition of fish were significantly affected by dietary lipid levels (P>0.05), while crude protein in carcass was not affected by dietary treatments (P<0.05) (Table 4). Crude lipid and energy contents in carcass of kutum had increased, when dietary lipid was increased from 50 g kg^{-1} lipid diets to 190 g kg^{-1} lipid diets, but moisture contents in carcass tended to decrease with the increase of dietary lipid level and same trend was observed for ash contents, meanwhile it was not significantly affected by dietary lipid level (P<0.05).

DISCUSSION

Dietary lipids are important nutrient affecting energy production in most of fish. They are well utilized by fish and essential for common growth and development.

However, fish are known to utilize protein preferentially to lipid or carbohydrate as an energy source.

The results of this trial based on feeding dietary lipid levels from 50 to 190 g kg^{-1} lipid diets, indicate that survival rate varied 95.00 to 99.58%. Likewise, it showed that the culture conditions were appropriate for the kutum fingerlings. The optimal dietary lipid level, estimated by polynomial regression analysis, for maximum growth of kutum fingerlings was 13.55% (Fig. 1). According to Shiau [23], the broken line model and regression methods are the methods frequently used to estimate dietary nutrient requirements of fish species. At the present study, it seems that the second order polynomial regression analysis to be more suitable because growth response of kutum was curvilinear. Our results showed that the increase in dietary lipid level from 50 to 120 g kg^{-1} lipid diets increased growth and slightly decreased thereafter with further increase in dietary lipid. All dietary treatments attained weight gain in excess of 200% of initial weight, except diets with 50 g kg^{-1} lipid diets. Diet containing 120 g kg^{-1} lipid diets with protein to energy ratio of 21.68 mg CP kJ^{-1} GE supported the best WG and SGR for kutum fingerlings. In this study, the highest specific
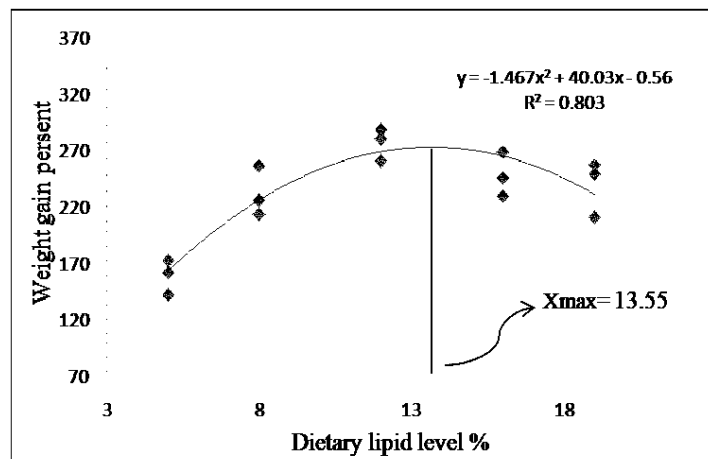

Fig. 1: Relationship between weight gain and dietary protein level for kutum fingerlings as described by second-polynomial regression.

growth rate of kutum (500 mg initial weight) was 2.54% day^{-1}, which is good score compared to other researches. For instance, Talebi Haghighi [24] registered maximum SGR of 2.78% day^{-1} for kutum fry (200 mg initial weight) fed a diet containing 468 g kg^{-1} protein and 80 g kg^{-1} lipid.

Some fish especially carnivorous species (most marine fish and marine crustacean) can employ higher dietary lipid for optimum growth rate [25-27]. On the other hand, some authors have reported that high dietary lipid level may be depressed fish growth [6, 27-30]. This reaction may be due to decrease in feed intake, excessive lipid deposition in visceral organs, to decrease in the efficiency or activity of digestive enzymes [31]. Likewise, Baker and Davies [32] reported that growth depression in high dietary lipid level could be due to increased malonaldehyde in oxidized lipid, which is poisonous to fish. In the present study demonstrated that the increase in dietary lipid level was collaborated with a reduction in feed intake, probably because fish eat to satisfy their energy requirements.

Feed and protein utilization efficiencies at the present study tended to improve with increasing level of dietary lipid. It implies that kutum fingerlings have a relatively good ability to utilize dietary lipids as energy sources. Appropriate quantities of lipid and/or carbohydrate sources (non-protein energy) in the fish diet can minimize catabolism of protein [33]. Lipid as energy source has been shown to provide a protein sparing effect in some fish [8-10]. Protein sparing intimates that lower dietary protein employed for energy to inclusion of lipid or carbohydrate (non-protein energy) sources has been displayed and elevate protein utilization for growth [34]. In many species like salmonid, up to 30% lipid diets have been proved to improve feed and protein efficiencies [35, 36].

In the present study, the FCR of experimental diets varied 1.77 to 3.50. The lowest dietary lipid level (50 g kg^{-1} lipid diets) produced the highest FCR, probably due to the intake of insufficient nutrient levels to promote growth. The best FCR was observed in fish fed diet containing 190 g kg^{-1} lipid diets.

The carcass composition of kutum was affected by dietary treatments except for ash and protein contents (Table 4). Body ash content of kutum showed no significant differences among all the experimental treatments. This effect has previously been observed in other fish species and aquatic animals [37-40]. The relationship between dietary lipids and deposition of fat in the fish body and tissue has been studied for several species. Very high dietary lipid levels might result in excessive lipid aggregation in the visceral cavity, tissue and liver and may consequently increased the whole body lipid concentrations [31]. The whole body lipid concentration of kutum used the high dietary lipid level was significantly higher than that of fish fed the low dietary lipid level. The carcass lipid of fish in this study was obviously increased corresponding to dietary lipid levels. A slight increase in body protein contents with the increase of dietary lipid level was also observed in kutum but, increase in body protein content was not significantly different among the treatments. In this study, whole body moisture was inversely related to whole body lipid content and a mutual relevance between lipid and moisture has been reported in many fish in general [3, 41].

CONCLUSION

Based on the results of this study, the maximum growth of kutum fingerlings was observed when fed the diet containing 120 g kg^{-1} lipid diets and the protein/energy ratio was approximately 21.68 g/MJ. Decreased growth and increased body fat were obvious when dietary lipid level increased from 120 to 190 g kg?1 lipid diets. From the polynomial regression analysis, the optimal dietary lipid requirement of kutum was estimated to be 13.55%. Further work to investigate the effects of dietary ingredients on enzyme excretion for kutum, *Rutilus frisii kutum*.

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