PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 10 (5): 415-423, 2011
ISSN 1680-5194

Effect of Varying Dietary Lipid Levels and Protein to Energy (P:E) Ratios on Growth Performance, Feed Utilization and Body Composition of Sub-adult Silver Pomfrets, *Pampus argenteus* (Euphrasen, 1788)

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Abstract: This study investigated the effect of varying levels of lipid and protein to energy (P:E) ratios on growth and feed utilization and body composition of sub-adult silver pomfret (*Pampus argenteus*). Duplicate groups of fish (average weight 98.2±0.2 g) were fed for 8 weeks three iso-nitrogenous experimental diets (49% protein) containing 12, 16 and 20% crude lipids with corresponding P:E ratio of 23.66, 22.62 and 21.60 for diets 1, 2 and 3 respectively. Mean body weight gain and specific growth rates of fish fed 16% and 20% lipid diets were significantly higher than that of 12% lipid diets. Daily feed intake was not affected by the dietary lipid levels, but there were significant differences in feed conversion ratio, protein efficiency ratio, apparent net protein utilization, energy and lipid retention values. Proximate analysis indicated that the lipid and fatty acid composition of whole body were affected by diets. Whole body of fish fed 16 and 20% lipid diets showed significantly ($p<0.05$) higher values for protein and lipid than fish fed 12% lipid diet. Higher Eicosapentaenoic Acid (EPA) and Decosahexaenoic Acid (DHA) were accumulated in fish body fed 16 and 20% lipid diets. This study demonstrated that there is no difference between the requirement for dietary lipid between juvenile and the sub-adult silver pomfrets and a dietary lipid level of 16% in a 49% protein diet corresponding to a P:E ratio of 22.6 is optimum for better growth, feed utilization and whole body composition of sub-adult silver pomfret.

Key words: Silver pomfret, dietary lipid, protein to energy ratio, growth, feed utilization

INTRODUCTION

Dietary lipids are important source of energy and essential fatty acids for fish. Moreover, dietary lipids acts as carrier of nutrients such as fat-soluble vitamin A, D and K (Watanabe, 1982). The optimization of dietary protein to energy ratio (P:E) has proven to have an important role on protein and energy utilization (Kaushik, 1994). Improper protein and energy level or their ratio will result in an increase in fish production cost and deterioration in water quality. Insufficient energy in diets causes protein wastes due to the increase of dietary protein proportion used for energy and produced ammonia can reduce water quality. Therefore, proper utilization of non-protein energy sources can increase protein and energy retention thereby decreasing the nitrogen excretion resulting a reduction in environmental output (Viola and Rappaport, 1979).

It is well established that for majority of the cultured fish species the protein efficiency utilization can be improved through the utilization of lipids and carbohydrate (Cho and Kaushik, 1990; Kaushik and Medale, 1994). However, a supplementation of lipid rather than carbohydrate as non-protein energy source is generally more effective for increasing energy level because lipid is readily metabolized by fish especially by carnivorous (NRC, 1993). The addition of lipids to a diet also contributes to effective utilization of dietary protein through the sparing effect in fish (Watanabe, 1982; De Silva *et al*., 1991; Skalli *et al*., 2004). However, fish are able to utilize dietary lipids up to a certain level, beyond which growth may be retarded owning to reduced feed consumption (Watanabe, 1982; Daniels and Robinsons, 1986; Ellis and Reigh, 1991). Again, a high lipid may cause an increase in body lipid deposition and affect carcass quality (Hillestad and Johnsen, 1994).

The silver pomfret, *Pampus argenteus* (Euphrasen) and their worldwide acceptance as an excellent food fish with high market demand has led to over-harvest of wild stocks in many areas. Recently Mariculture and Fisheries Department (MFD) of Kuwait Institute for Scientific Research (KISR), Kuwait has been successful in the spawning of captive silver pomfret (James and Almatar, 2007) and presently research is being carried out to develop a commercial culture technology for silver pomfret. For cost effective feed development it is important to define an optimal dietary lipid level for silver pomfret. In our previous study using juvenile pomfret with varying levels of dietary lipids (12-20%) showed significantly ($p<0.05$) higher weight gain and specific growth rates in silver pomfret fed diet containing 16%

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lipid and a protein to energy (P:E) ratio of 22.6 (mg protein/kJ gross energy). A further increase in lipid level above 16% did not produce any improvement in the growth and feed utilization (Hossain *et al*., 2011). So far almost all the lipid studies conducted with different species of fish were either with fry or juveniles and studies using sub-adult fish is scarce. To compare the effects of varying dietary lipids between the juveniles and sub-adults, in the present study we evaluated the effect of varying levels of lipids and P:E ratios on the growth performance, feed utilization and body composition of sub-adult silver pomfrets.

MATERIALS AND METHODS

Experimental system: The experiment was carried out using 2-m^3 capacity round fiber glass tanks each containing about 1800 L of water. The tanks were painted black internally and the bottom had a 2° slope towards a 5-cm diameter central drainage stand pipe which provided water drainage from the bottom. The tanks were covered with nets to prevent the fish from jumping out. Filtered and UV treated sea water and ground water were mixed and flowed through the tanks of an open flow-through system at the rate of 10 L/min. The water temperature in experimental tanks were maintained at 28.0°C using in-line heater "Aqualogic" (model HTI-8-220, 8 KW from Aquatic Ecosystems, USA) in the main inflow line. Continuous aeration was provided by two air stones using 1.5-inch PVC air-lift pipes inside the tanks to avoid air bubbles in the culture system. Fluorescent lights were used to provide a light intensity of 400-480 lux above the water surface and natural photoperiod of 12 h light and 12 h dark was maintained throughout the study period.

Source of fry and acclimation: Nine months old sub-adult silver pomfrets (average weight 98.2 g) were obtained from MFD, KISR hatchery which were originated the from eggs collected from the wild spawners during 2009 spawning season. They were reared on a mixture of commercial feeds (50% protein, Biomar, France and 55% protein, GEMMA Micro 300, Skretting, England) before used in this study. For handling the fish while stocking and sampling 3 ppm of the tranquilizer "quinaldine" (Argent Chemical Laboratory, Redmond, USA) was used. Prior to the initiation of the experiment, fish were randomly stocked in the respective experimental tanks and acclimated to the experimental condition for one week.

Feed formulation and diet preparation: The diets used in this study were similar to those used in our previous study with silver pomfret juveniles (Hossain *et al*., 2011). The experimental diets were formulated to contain 12, 16 and 20% lipid level with corresponding P:E ratio of 23.66, 22.62 and 21.60 for diets 1, 2 and 3 respectively. The diets were formulated to contain in the order of 49% protein (Hossain *et al*., 2010). The experimental diets were prepared using a mixture of three commercial diets (Gemma diamond 0.8 mm, Gemma Micro 300 and Biomar 3 mm). Prior to formulation of experimental diets, the proximate composition of these commercial diets were analyzed (Table 1).

Fish oil from menhaden (Sigma-Alrich Co., Germany) was used as supplemental lipid. Diets were also fortified with vitamin premix (1%), mineral premix (1%), binder (1%), nucleosac (0.5%) and probiofeed (0.5%). In diets 1 and 2, wheat flour and alpha-cellulose were used as filler or bulking material to keep the dietary lipid content at a desired level. In order to prepare diets, all the dietary ingredients were finely ground and sieved to pass through 0.5 mm mesh. All dietary ingredients including the vitamin and mineral premixes were weighed as per formulae (Table 2) and mixed thoroughly with an electrical mixer machine. During mixing oil was poured gradually and mixed thoroughly to ensure homogeneity. The mixed feed was stored in air-tight container at -20°C until used for feeding. Before daily feeding each feed mixture was moist pelleted by adding about 35-40% fresh water and again made into paste/dough by hand before presentation to fish. Addition of agar as binder resulted in a dough with suitable soft and sticky consistency which helped to adhere the feed at the bottom of the bowl (feeding tray) for more than two hour. This reduced the rate of disintegration of the feed. The diets were subjected to proximate and fatty acid analysis and the results are shown in Table 3.

Experimental procedure, feeding and sampling of fish: Circular fiber glass tanks of 2-m^3 capacity containing about 1800 L of water were randomly divided into three dietary groups each with two replicates. It may be mentioned that due to lack of physical facilities available, it was not possible to use more than two replicates. Sub-adult silver pomfrets (initial mean body weight 98.2±0.2 g) were randomly distributed at a density of 10 fish per tank. Since the fish are reluctant to take pelleted feeds, they were offered the diets in paste/dough form. Fish were offered each diet to apparent satiation thrice daily, 7 days a week. The feed was offered at 9.00 and 14.00 h and 18.00 h in white plastic bowls placed at the bottom of the tanks. As mentioned earlier, the feeds supplied in paste/dough form had a soft but sticky consistency which helped to adhere the feed at the bottom of the feeding bowl resulting in reduced disintegration of feeds. However, complete prevention of disintegration and nutrient leaching could not be ascertained in such form of feeding used. Two hours after feeding the bowls left with any uneaten feed were lifted very carefully, filtered on fine-meshed (50 μ) plankton net and dried in an oven at 60°C to calculate the amount of feed consumed. Mortality of fish if any was recorded.

Table 1: Proximate composition of feeds (% dry matter basis) used in experimental diet formulation

Feed/ingredients	Dry matter	Crude protein	Lipid	Ash	Crude fibre	NFE[a]
GEMMA diamond[b]	91.05	58.00	14.50	9.85	1.10	16.55
GEMMA Micro-300[c]	91.10	55.00	15.00	13.50	2.05	14.45
BIOMAR[d]	90.67	50.97	12.38	7.23	4.45	24.97

[a]Nitrogen free extract calculated as 100 - %(crude protein + lipid + ash + crude fibre).
[b]Skretting, Cheshire, England (particle size-0.8 mm)
[c]Skretting, Cheshire, England (particle size-300 micron)
[d]Biomar, France (particle size-3 mm)

Table 2: Formulation (%) of the experimental diets

Ingredients	Diet-1 (12% lipid)	Diet-2 (16% lipid)	Diet-3 (20% lipid)
GEMMA (Diamond)	40.00	40.00	40.00
GEMMA Micro-300	30.00	30.00	30.00
Biomar feed (3mm)	18.00	18.00	18.00
Fish oil from manhaden[a]	-	4.00	8.00
Vitamin premix[b]	1.00	1.00	1.00
Mineral premix[c]	1.00	1.00	1.00
Nucleosac[d]	0.50	0.50	0.50
Probiofeed[e]	0.50	0.50	0.50
Agar (binder)	1.00	1.00	1.00
Wheat flour	5.00	2.00	-
alpha-cellulose	3.00	2.00	-
Total	100.00	100.00	100.00

[a]From Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany.
[b]Vitamin premix contained the following mixed with cellulose (g/kg mix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; pantothenic acid, 5.0 g; inositol, 100 g; biotin, 0.30 g; folic acid, 0.75 g; para-amino benzoic acid, 2.5 g; choline, 200 g; niacin, 10.0 g; cyanocobalamine, 0.005 g; retinol palmitate (Vit-A), 100,000IU; alpha-tocopherol acetate (Vit-E), 20.1 g; ascorbic acid, 50.0 g; menadione, 2.0 g, cholecalciferol (Vit-D), 500,000 IU.
[c]Mineral premix contained the following ingredients (g/kg): calcium orthophosphate ($2H_2O$), 727.78; magnesium sulfate ($7H_2O$), 127.50; sodium chloride, 60.00; potassium chloride, 50.00; ferrous sulfate ($7H_2O$), 25.00; zinc sulfate ($7H_2O$), 5.50; manganese sulfate ($4H_2O$), 2.5375; copper sulfate ($5H_2O$); 0.7875; cobalt sulfate ($7H_2O$), 0.4775; calcium iodide, 0.2950; chromium chloride, 0.1275.
[d]Nucleosac-Antistress-growth promoter (Zymonutrient Pvt. Ltd, Karnataka, India).
[e]Probiofeed-a propbotics from Zymonutrient Pvt. Ltd, Karnataka, India

Besides weighing at the beginning and at the end of the feeding trial, the fish were weighed bi-weekly to observe the growth rate. All the fish in the experimental tanks were weighed individually during each sampling using a digital sensitive balance (Model SBC-61, Scaltec Instruments, Gottingen, Germany with 0.01 g sensitivity). At the beginning of experiment ten fish from the stock were collected and frozen at -80°C, freeze dried and finely ground for subsequent proximate and fatty acid analysis. At the end of the experiment, three fish from each replicate tank were sampled in the same manner for whole-body proximate and fatty acid analysis. The feeding trial was conducted for 8 weeks.

Water quality parameters: The water quality parameters such as water temperature, dissolved oxygen, pH were monitored daily through the experimental period. The total ammonia and salinity were measured weekly. All the parameters are found to be suitable for fish growth and survival. The ranges were: temperature 28.0-28.5°C, pH 7.2-8.1, dissolved oxygen 5.85-6.65 mg/L, salinity 38.0-40.0 ppt. and total ammonia 0.03-0.06 mg/L.

Analytical methods and statistical analysis: The proximate composition of fish samples, commercial and experimental diets was analyzed in triplicate according to the following procedure (AOAC, 2000): moisture content by drying in an oven at 105°C for 24 h; crude protein content (N x 6.25) by the Kjeldahl method using an Auto Kjeldahl System (Kjeltec™ 2300 Foss Tecator AB, Hoganas, Sweden), lipid by ether extraction (Soxtec System HT6, Tecator AB, Hoganas, Sweden), ash by incineration in a muffle furnace at 600°C for 6 h. Nitrogen free-extracts were calculated from the difference after determining crude fiber by digestion with 1.25% H_2SO_4 and 1.25% NaOH solutions (Fibertec System M, Tecator AB, Hoganas, Sweden). Lipid content of samples for fatty acid analysis was extracted by the Bligh and Dyer (1959) method. Fatty acid composition was determined by preparing methyl esters and analyzing them by gas chromatography (AOCS, 1992). An HP 6890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with an Chromapack column (CP-Sil 88 50 meter, ID 0.25 mm, Varian Inc, Palo Alto, CA, USA) was used for the analysis. The oven temperature program was as follows: initial temperature 50°C and increased 10°C per minute to final temperature 235°C, hold for 7 min. Fatty acid methyl ester standard mixture comprising 25 different fatty acids (ranging from C8 to C22:6) were obtained from Altech Associates, Deerfield, USA. Fatty acids were identified by comparison of retention times

Table 3: Analyzed proximate and fatty acid (% of total fatty acids) composition of the experimental diets (% dry matter basis)

	Diet-1 (12% lipid)	Diet-2 (16% lipid)	Diet-3 (20% lipid)
Proximate composition			
Dry matter	92.08	92.44	92.83
Crude protein	49.22	49.41	49.24
Lipid	12.37	16.51	20.32
Ash	9.37	9.21	9.07
Crude fibre	4.08	3.62	3.01
NFE[a]	24.96	21.25	18.36
Gross energy (kJ/g)[b]	20.80	21.84	22.80
P:E ratio[c]	23.66	22.62	21.60
Fatty acids[d]			
C14	5.92±0.12	7.21±0.14	7.35±0.14
C15	0.44±0.03	0.58±0.03	0.61±0.03
C16	31.17±0.51	30.32±0.48	28.00±0.44
C17	0.60±0.06	0.42±0.06	0.44±0.05
C18	6.19±0.12	5.98±0.15	6.07±0.12
C20	0.45±0.02	0.29±0.02	0.34±0.00
C16:1	3.43±0.09	5.09±0.10	5.96±0.05
C17:1	0.29±0.00	0.44±0.00	0.57±0.02
C18:1n-9	15.13±0.14	15.27±0.11	15.58±0.09
C20:1	4.62±0.11	3.79±0.08	3.83±0.07
C22:1n-9	3.21±0.7	2.54±0.06	2.70±0.09
C24:1	Nd	Nd	Nd
C18:2n-6	16.37±0.14	12.60±0.14	11.57±0.10
C18:n-3	1.51±0.09	1.62±0.07	1.76±0.04
C18:3n-6	Nd	Nd	Nd
C20:3 n-3	0.29±0.03	0.45±0.04	0.48±0.01
C20:3n-6	0.11±0.002	0.19±0.00	0.31±0.01
C20:4n-6	Nd	Nd	Nd
C20:5n-3, EPA	6.25±0.09	7.76±0.10	8.41±0.09
C22:6n-3, DHA	4.02±0.05	5.55±0.04	6.02±0.04
ΣSFA[e]	44.47	44.70	42.81
ΣMUFA[e]	26.68	27.13	28.64
ΣPUFA[e]	28.55	28.17	28.55
Σn-3	12.07	15.38	16.67
Σn-6	16.48	12.79	11.88
n-3/n-6 ratio	0.73	1.20	1.40

[a]Nitrogen free extract (total carbohydrate) calculated as 100-% (crude protein + lipid + crude fibre + ash).
[b]Estimated according to NRC (1993) using the values of 23.6, 39.5 and 17.2 kJ/g for crude protein, lipid and total carbohydrate respectively.
[c]Protein to energy ratio in mg protein kJ/g of gross energy.
[d]Values are mean±SD (n = 3).
[e]SFA: Saturated Fatty Acid; MUFA: Mono-saturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; Nd: Not detected

Table 4: Growth performance and feed utilization of juvenile silver pomfret fed diets containing varying lipid levels and P:E ratios[1]

Parameters	Diet 1 (12% lipid)	Diet 2 (16% lipid)	Diet 3 (20% lipid)
Mean initial weight (g)	98.00±0.57[a]	98.40±1.70[a]	98.30±2.40[a]
Mean final weight (g)	109.00±0.80[a]	116.60±1.20[b]	116.20±2.60[b]
Mean weight gain (g)	11.00±0.28[a]	18.20±0.60[b]	17.90±2.40[b]
DWG (g/fish/day)[2]	0.20±0.01[a]	0.33±0.02[b]	0.32±0.02[b]
SGR (%/day)[3]	0.19±0.01[a]	0.30±0.02[b]	0.30±0.02[b]
DFI (%)[4]	2.31±0.11[a]	2.25±0.11[a]	2.22±0.15[a]
FCR[5]	2.45±0.10[b]	2.18±0.09[a]	2.18±0.10[a]
PER[6]	0.80±0.03[a]	0.94±0.03[b]	0.94±0.01[b]
ANPU (%)[7]	11.64±0.65[a]	26.89±0.89[b]	26.32±1.25[b]
ER (%)[8]	18.75±1.03[a]	43.58±1.17[b]	42.23±0.54[b]
LR (%)[9]	33.88±0.94[a]	84.10±1.54[b]	82.17±1.33[b]
Survival (%)	95.00±0.00[a]	95.00±0.00[a]	95.00±0.00[a]

[1]Values (mean±SD) in a row with different superscripts are significantly different determined by Tukey's test ($p<0.05$).
[2]DWG: Daily Weight Gain (g/fish/day).
[3]Specific growth rate =100 x (ln [final body weight] - ln [initial body weight]/experimental period (days).
[4]DFI: Daily Feed Intake (%) = Feed intake (dry matter) x 100/[initial fish weight + final fish weight) x days fed/2].
[5]FCR: Feed Conversion Ratio = Dry feed fed/live weight gain.
[6]PER: Protein Efficiency Ratio = Live weight gain/crude protein fed.
[7]ANPU: Apparent Net Protein Utilization = (final fish body protein - initial fish body protein)/(total protein fed) x 100.
[8]ER: Energy Retention (%) = 100 x (energy gain, kJ/energy intake, kJ).
[9]LR: Lipid Retention (%) = 100 x (lipid gain/lipid intake)

Table 5: Whole body proximate and fatty acid composition of silver pomfret fed diets containing varying dietary lipid levels and P:E ratios[1]

Components	Initial (all fish)	Diet 1 (12% lipid)	Diet 2 (16% lipid)	Diet 3 (20% lipid)
Proximate composition (% fresh matter basis)				
Moisture	77.51	76.20±0.26^{b}	71.16±0.40^{a}	71.09±0.0.18^{a}
Crude protein	15.10	16.01±0.20^{a}	17.22±0.24^{b}	17.10±0.09^{b}
Lipid	4.12	4.79±0.24^{a}	8.88±0.26^{b}	9.05± 0.21^{b}
Ash	2.74	2.78±0.07^{a}	2.68±0.06^{a}	2.69±0.06^{a}
Fatty acid composition (% of total fatty acids)				
C14	6.22	5.93±0.10^{ab}	5.50±0.08^{a}	6.01±0.17^{b}
C15	0.45	0.24±0.1^{b}	0.21±0.01^{ab}	0.17±0.02^{a}
C16	24.16	19.20±0.65^{a}	20.08±0.23^{a}	19.97±0.29^{a}
C17	0.66	1.12±0.11^{a}	0.94±0.04^{a}	0.97±0.07^{a}
C18	5.44	4.16±0.55^{a}	4.31±0.28^{a}	3.70±0.32^{a}
C20	0.29	0.36±0.04^{a}	0.45±0.04^{a}	0.41±0.08^{a}
C21	2.34	3.49±0.49^{b}	2.05±0.17^{a}	1.86±0.29^{a}
C16:1	5.34	6.42±0.44^{a}	7.29±0.09^{a}	7.39±0.13^{a}
C17:1	0.30	0.84±0.04^{a}	0.91±0.05^{a}	0.86±0.09^{a}
C18:1n-9	22.36	23.39±0.21^{b}	21.15±0.38^{a}	20.54±0.28^{a}
C20:1	5.45	3.82±0.40^{a}	4.07±0.33^{a}	4.48±0.09^{a}
C22:1n-9	6.42	7.81±0.33^{b}	5.19±0.59^{a}	5.34±0.33^{a}
C24:1	0.68	1.77±0.23^{a}	1.47±0.08^{a}	1.39±0.03^{a}
C18:2n-6	11.66	13.18±0.80^{a}	14.99±0.17^{b}	14.76±0.27^{b}
C18:3n-3	0.38	0.18±0.03^{a}	0.17±0.01^{a}	0.15±0.02^{a}
C18:3n-6	Nd	Nd	Nd	Nd
C20:3n-3	0.27	0.18±0.03^{a}	0.17±0.01^{a}	0.15±0.02^{a}
C20:3n-6	Nd	Nd	Nd	Nd
C20:4n-6	Nd	Nd	Nd	Nd
C20:5n-3, EPA	3.33	2.74±0.11^{a}	3.69±0.73^{ab}	4.50±0.18^{b}
C22:6n-3, DHA	3.41	4.16±0.28^{a}	6.46±0.99^{b}	6.57±0.16^{b}
ΣSFA[2]	39.56	34.50±0.56^{a}	33.53±0.38^{a}	33.07±0.32^{a}
ΣMUFA[2]	40.55	44.04±1.90^{a}	40.02±1.45^{a}	39.00±0.27^{a}
ΣPUFA[2]	19.05	20.65±1.20^{a}	25.66±1.90^{b}	26.26±0.21^{b}
Σn-3	7.39	7.08±0.42^{a}	10.42±1.88^{ab}	11.11±0.12^{b}
Σn-6	11.66	13.18±0.78^{a}	14.99±0.11^{b}	14.76±0.21^{b}
n-3/n-6 ratio	0.63	0.54±0.03^{a}	0.69±0.13^{a}	0.75±0.03^{a}

[1]Values (mean±SD) in row with different superscripts are significantly different determined by Tukey-Kramer's test ($p<0.05$).
[2]SFA: Saturated Fatty Acid; MUFA: Mono-saturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids. Nd: Not detected

with a mixture of standards containing all the fatty acids identified in this study. Each fatty acid was quantified by calculating its peak area relative to the total peak area. These values are referred to as fatty acid content (%) throughout the paper. An estimated amount of each fatty acid was calculated from the lipid content and fatty acid content, which approximately corresponds to g fatty acid per 100 g lipid.

Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Apparent Net Protein Utilization (ANPU %) were calculated according to Castell and Tiews (1980). Data were subjected to one way ANOVA using MSTATC package and if significant differences were found, Tukey's test was used to rank the group. Statistical analyses were carried out using a package Super ANOVA, ver.1.11, Abacus Concepts, Berkley, CA, USA.

RESULTS

Growth performances and feed utilization of sub-adult silver pomfrets fed experimental diets are presented in Table 3. Fish fed diets 2 and 3 resulted in the significantly ($p<0.05$) higher weight gain, daily weight gain (DWG g/fish/day) and specific growth rate (SGR%/day) than those of fish fed diet 1. However, there was no significant differences between the weight gain, DWG and SGRs of fish fed diets 2 and 3. There was no significant difference ($p>0.05$) among the Daily Feed Intakes (DFI) between the dietary groups although fed diets 2 and 3 showed significantly ($p<0.05$) lower Food Conversion (FCR) values than that of diet 1. The Protein Efficiency Ratio (PER) values showed the similar trend like those of FCRs which ranged between 0.80 and 0.94. Fish fed diets 2 and 3 resulted in significantly higher Apparent Net Protein Utilization (ANPU%), Energy Retention (ER%) and Lipid Retention (LR%) values compared to those fed diet 1. However, there was no significant difference between the ANPU, ER and LR values of fish fed diets 2 and 3.

Whole body proximate composition and fatty acid profile of sub-adult silver pomfrets at the beginning and end of the experiment is given in Table 5. Fish fed diet 1 with 12% lipid had significantly ($p<0.05$) higher whole body moisture and lower lipid than those fed diet 2 (16% lipid)

and diet 3 (20% lipid). There was no significant difference between the whole body protein contents of fish fed diets 2 and 3 which were significantly higher than that of fish fed diet 1. Increase of dietary levels of lipid significantly affected the whole body lipid level of fish. Fish fed diet 3 had higher levels of lipid (9.05%) than those fed diet 2 (8.88%) although not statistically different. However, there was no significant differences in whole body ash contents among different dietary groups which ranged between 2.68-2.78%.

Among the whole body Saturated Fatty Acids (SFAs), C16:0 was the dominant SFA in all dietary groups amounting 19.20-20.08% of the total fatty acids. Among Monosaturated Fatty Acids (MUFAs) C18:1n-9 was the dominant one followed by C16:1 and C22:1n-9. Fish fed diet 1 had significantly ($p<0.05$) higher C18:1n-9 and C22:1n-9 than those fed diets 2 and 3. Fish fed diets 2 and 3 had significantly higher DHA (docosahexaenoic acid) content than those fed diet 1 while there were no significant differences in Eicosapentanoeic Acid (EPA) contents of fish fed diets 1 & 2 and 2 & 3 respectively. **There were no significant differences in Σ SFA and Σ MUFA contents between different dietary groups. However, the ΣPUFA, Σn-3, Σ n-6 in fish fed diets 2 & 3** were significantly ($p<0.05$) higher than those fed diet 1.

DISCUSSION

The observed growth of sub-adult silver pomfret was much slower than that observed in our previous study (Hossain *et al*., 2011) using similar diets, where juveniles of 34 g reached 85 g in 4 months. This discrepancy may be attributed to the larger initial size of the fish used in the present study as Specific Growth Rates (SGR) decreases with increasing fish sizes. The SGR of fish fed diet 2 and 3 in the present study was only 0.30 whereas it was 0.74 and 0.64 for silver pomfret juveniles fed diets 2 and 3 respectively in our previous study.

Number of studies with fish have shown that increase of dietary lipid level improves growth, feed and protein efficiency as it spares proteins that could otherwise have been catabolized and used as energy source (Hillestad and Johnsen, 1994; Lee *et al*., 2002; Skalli *et al*., 2004). In the contrary, however, some authors observed no protein sparing effect of lipid (McGoogan and Gatlin, 1999; Thoman *et al*., 1999; Yildirim-Aksoy *et al*., 2007). In general, fish have an optimum level of dietary lipids over which dietary fat can cause growth depression (Tidwell *et al*., 1996; Weatherup *et al*., 1997; Du *et al*., 2005; Lopez *et al*., 2006). This observation was confirmed in the present study in which 16% lipid diet was the most suitable for sub-adult silver pomfret in terms of growth compared to 12 and 20% lipid diet. This is in agreement with our previous study with juvenile silver pomfret (Hossain *et al*., 2011). Similar observations have been found with other species such as Asian sea bass (*Lates calcarifer*), white sea bass (*Atractoscions nobilis*) and meager (*Argyrosomus regius*) which showed higher growth rates when fed diets containing 15% lipid compared to 13 or 23% lipid, 19.5 or 21.5% lipid or 13 or 21% lipid, respectively (Williams *et al*., 2003; Lopez *et al*., 2006; Chatzifotis *et al*., 2010).

The fish fed higher lipid (20%) diet did not improve growth and PER which indicated that excessive lipid can results in higher fat accretion and impaired growth performance. Similar results have also been reported for other species such as gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and common dentex (*Dentex dentex*) (Company *et al*., 1999; Peres and Oliva-Teles, 1999; Espinos *et al*., 2003). Most of the studies attributed the reduced growth of fish fed high lipid diets to the excessive energy that can cause reduced feed consumption resulting in growth retardation (Kaushik and Medale, 1994; Upatsch *et al*., 2001). On the other hand, some studies have shown that feed intake seems to adjust to protein intake rather than energy intake (Santinha *et al*., 1999; Peres and Oliva-Teles, 1999; Du *et al*., 2005). In the present study, there was no significant differences in daily feed intake among the dietary groups which also support the above findings. The better growth of fish fed diets 2 and 3 may be attributed to the better FCR. Increasing the lipid content up to certain level with diets having same protein content in the present study led to an improvement in growth performance and feed utilization suggesting that protein may be utilized for growth rather than energy. This conclusion is supported by the better PER and ANPU values observed with increased dietary lipid levels in our present study.

The influence of changes in dietary P:E ratios on growth and protein utilization has been demonstrated in several fish species of fish. In the present study, a P:E ratio of 22.66 with 16% dietary lipid was found optimum for sub-adult silver pomfret. This P:E value is similar to the P:E ratio of 21-22 reported for European sea bass (Dias *et al*., 1998; Peres and Oliva-Teles, 1999) but slightly lower than reported optimum values of 23.7 for sunshine bass (Webster *et al*., 1995) and 25.9 for Japanese sea bass (Ai *et al*., 2004). However, the optimum P:E ratios in fish may vary depending on the species, experimental P:E levels and the dietary ingredient used in feed formulation (Ai *et al*., 2004).

The proximate composition of cultured fish is affected by exogenous and endogenous factors (Shearer, 1994). The correlation between dietary lipid and body lipid has been extensively studied. Too much dietary lipid may results in excessive fat deposition (Catacutan and Coloso, 1995; Weatherup *et al*., 1997; Peres and Oliva-Teles, 1999; Lopez *et al*., 2006). In the present study, the whole body lipid levels of fish fed diet 2 and 3 were significantly higher than that of fish fed diet with lower

lipid. Similar results have also been reported for other species such as red drum, *Scaenops ocellata* (Ellis and Reigh, 1991), European sea bass (Peres and Oliva-Teles, 1999) gilthead sea bream (Vergara *et al*., 1996) cobia, *Rachycentron canadum* (Wang *et al*., 2005), grass carp, *Ctenopharyngodon idella* (Du *et al*., 2005) and Atlantic halibut, *Hippoglossus hippoglossus* (Martins *et al*., 2007).

It is well known that the FA composition of tissues is determined mainly by their dietary lipid and that the marine fish have a specific requirement for EPA and DHA (Sargent *et al*., 1999; Tocher, 2003) Marine fish generally show a good growth when EPA and DHA are supplied at a combined rate of between 0.8 and 2.0% (NRC, 1993). The combined dietary level of EPA and DHA in the present study (1.2-2.8%) is higher than the requirement (0.8-0.9%) reported for juvenile flat fish such as turbot, *Scophthalmus maximus* (Gatesoupe *et al*., 1977) and starry flounder, *Platichthys stellatus* (Lee *et al*., 2003). Fatty acid deposition of sub-adult silver pomfret in the present study was reflective of levels present in each experimental diet. Marked changes in the whole body tissues of fatty acid composition as a response of increasing dietary lipid, particularly EPA and DHA rather than linolenic acid (C18:3n-3) was observed in the present study indicating that direct incorporation was likely. DHA is reported to have more important functions for growth and development of fish than EPA. Compared to the initial whole body composition, the deposition of DHA was much higher than the EPA. Similar results of higher incorporation of DHA into body tissue have also been observed in our previous study with silver pomfret juvenile (Hossain *et al*., 2011) and in starry flounder (Lee *et al*., 2003). Robin *et al*. (2003) also reported that DHA is the major PUFA of fish membranes and preferentially incorporated in turbot. In conclusion, the result of the present study indicated that that there is no difference between the requirement for dietary lipid between juvenile and the sub-adult silver pomfrets and a dietary lipid level of 16% in a 49% protein diet corresponding to a P:E ratio of 22.6 is optimum for better growth, feed utilization and whole body composition of juvenile and sub-adult silver pomfret.

ACKNOWLEDGEMENTS

This research was funded by the project "Culture of Zobaidy (*Pampus argenteus*) in Kuwait" Phase II (Project No. FM 046K) supported by Kuwait Foundation for the Advancement of Sciences (KFAS), Arab Fund for Economic and Social Development (AFESD) and KISR, Kuwait. Thanks are due to Ms Sharifa Barak Yaseen of MFD, KISR for her assistance with the proximate analysis. The authors would like to thank Mr. Nisar Ahmed of Central Analytical Laboratory (CAL), KISR for fatty acid analysis of feed and fish samples.

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