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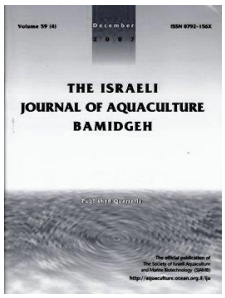

Protein/energy ratio and HUFA content in the diet of *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings: Effect on growth and flesh quality

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Key words: protein energy ratio, growth, *Pangasianodon hypophthalmus*, specific growth rate, flesh quality

Abstract

A 60 day feeding trial was conducted to study the effect of varying levels of protein/energy (P/E) ratio and highly unsaturated fatty acid (HUFA) supplemented diet on growth and flesh quality parameters of *Pangasianodon hypophthalmus* fingerlings. One hundred and eighty fingerlings of uniform weight (4.32±0.08g) were randomly distributed into five treatment groups with three replications. The five different treatment groups were: Control - T0H0 (basal feed+117 mg/kcal, P/E ratio); T1H0 (basal feed +100 mg/kcal, P/E ratio); T2H0 (basal feed +133 mg/kcal, P/E ratio); T3H1 (basal feed +100 mg/kcal, P/E ratio+1% HUFA) and T4H1 (basal feed + 133 mg/kcal, P/E ratio+1% HUFA). Significantly higher ($P<0.05$) weight gain (WG) %, specific growth rate (SGR), and feed conversion efficiency (FCE) were observed in the T4H1 and T2H0 groups. Feed conversion ratio (FCR) and protein efficiency ratio (PER) were lowest in the T4H1 group. T3H1 and T4H1 groups manifested significantly higher ($P<0.05$) value for flesh quality indices such as springiness, adhesiveness, gumminess, and chewiness. Lowest hardness value was found in T4H1 group. Significantly higher ($P<0.05$) EPA and DHA deposition in fish muscle was observed in T4H1 group. Protease activity was higher in T4H1 group followed by T2H0 group and lowest in T1H0 group. Amylase activity was lower in T2H0 and T4H1 groups. Overall results revealed that P/E ratio of 133 mg/kcal with additional supplementation of 1% HUFA in the diet enhances growth and improves flesh quality of *P. hypophthalmus* fingerlings.

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Introduction

An important aspect in fish culture is the transformation of dietary protein to protein deposited in the body of the fish. A considerable proportion of dietary protein (amino acids) is catabolized as energy to maintain metabolism (NRC, 1993). The proportion of dietary protein utilized as energy fuel is related to the proportion of non-protein energy in dietary digestible energy. As protein is the most expensive component in the fish diet, efficient use of dietary protein is imperative to minimize production costs.

Inclusion of non-protein energy sources has been shown to lower use of dietary protein for energy and increase protein utilization for growth, a process known as "protein sparing" (El-Sayed and Teshima 1992; Morais et al., 2001; Yigit et al. 2002), while excessive non-protein energy results in reduced feed intake (Lovell, 1989), unwanted fat deposition inhibits utilization of other nutrients and eventually results in reduction in growth rate (Winfree and Stickney, 1981). Therefore, an optimal dietary protein to energy (P/E) ratio should be considered when formulating feed of cultivable species; 90-110 mg protein/kcal of digestible energy (DE) has been found to be optimum for maximum protein gain in channel catfish fingerlings (Mangalik, 1986). Studies have been conducted to determine the optimal protein energy ratio for better growth of some cultured fish (Catacutan and Coloso, 1995; Hernandez et al., 2001).

In addition to growth being important, flesh quality is also extremely important as the market price of farm produce depends largely on meat quality. Flesh quality is influenced by several factors such as feed composition, breeding season, and filleting methodology (Roth et al., 2009). Supplementation of dietary EPA and DHA in the feed has been shown to enhance the flesh quality. The addition of 1% linolenic acid to European catfish (*Silurus glanis*) formulated feed has beneficial effects on fish growth indicators and meat quality (Bogut et al., 1997).

Asian catfish, *Pangasianodon hypophthalmus* is a native of the Mekong Delta and is widely cultured in ponds in the South-East Asian countries (Hung et al., 2004). This species, promoted for large scale aquaculture production in India, is of great interest to fish farmers for intensive farming of this species. The huge production potential of *P. hypophthalmus* may be attractive for both domestic and international markets. However, in order to enhance the selling price, flesh quality needs to improve as the species is currently sold at cheaper prices in both of these markets. Although some literature is available on the nutritional requirements of *P. hypophthalmus* (Hung *et al.,* 2004: Liu *et al.,* 2011), information on dietary optimum protein energy ratio with HUFA for growth and improvement of flesh quality is scarce.

The present investigation was designed to elucidate the effect of varying levels of P/E ratio diet supplemented with HUFA in terms of growth and flesh quality responses of *P. hypophthalmus* fingerlings.

Materials and Methods

Experimental site and experimental animals. The experiment was conducted in the wet laboratory of the Aquaculture division of the Central Institute of Fisheries Education (CIFE), Mumbai, India, and the laboratory analysis was carried out at the Fish Nutrition, Biochemistry, and Physiology (FNBP) division of CIFE, Mumbai. Proximate and fatty acid analysis was carried out in the FNBP division while texture analysis was carried out in the Fish Processing division of CIFE, Mumbai.

P. hypophthalmus fingerlings (average weight 4.32±0.08 g) were procured from the Murbad fish farm Maharashtra, India, in March, 2012. They were transported, stocked in a cement tank (1000 l capacity) and left undisturbed overnight. The following day, fish were given a mild salt treatment in order to ameliorate the handling stress. The stock was acclimatized under aerated conditions for 20 days. During acclimation, fish were fed a controlled diet (35% CP, P/E ratio 117mg/kcal). A compressed air pump provided round the clock aeration and water was exchanged every alternate day. Throughout the experimental period the physico-chemical parameters of the water were within the optimum range (dissolved oxygen: 6.56–7.1 mg/l; pH:7.25–7.8; temperature: 26.6–28.2^{o}C; alkalinity 46-58 mg/l and hardness 48-64 mg/l) (Gupta et al., 2012).

Experimental design, feed, and feeding. One hundred eighty *P. hypophthalmus* fingerlings were randomly distributed into five treatment groups in triplicate in a completely randomized design (CRD). The experimental rearing system consisted of 15 uniform rectangular fiber reinforced plastic tanks (150 l capacity). The volume of the water in each tank was maintained at 100 l throughout the experimental period.

The fish were divided into five different treatment groups and five experimental diets were prepared (Table 1):
Control T0H0 (basal feed+117 mg/kcal, P/E ratio);
T1H0 (basal feed +100 mg/kcal, P/E ratio);
T2H0 (basal feed +133 mg/kcal, P/E ratio);
T3 H1 (basal feed +100 mg/kcal, P/E ratio+1% HUFA) and
T4 H1 (basal feed +133 mg/kcal, P/E ratio+1% HUFA).

Table 1. Proximate composition of the experimental diets.

Ingredients (%)	*Diets*				
	T_0H_0	T_1H_0	T_2H_0	T_3H_1	T_4H_1
Fish Meal	22	16	34	16	34
Soybean meal	26	22	24	22	24
Mustard oil cake	14	14	13	14	13
Ground oil cake	8	5	5.7	5	4.8
Wheat flour	9.7	14	7	14	7
Rice powder	6	13	4	13	4
Corn flour	7	7.7	3	6.8	3
Sunflower oil	4	5	6	5	6
B.H.T[1]	0.3	0.3	0.3	0.3	0.3
C.M.C[2]	1	1	1	1	1
Vitamin premix[3]	2	2	2	2	2
HUFA[4]	-	-	-	1	1
Proximate composition of different experimental diets					
Crude Protein (%)	34.74	30.54	39.55	29.73	39.81
Crude Fat (%)	3.64	3.90	1.91	3.91	1.86
DE[5](kcal/100g)	299	299.4	300	299.4	300
P/E[6] ratio(mg/kcal)	117	100	133	100	133

[1]B.H.T (butylated hydroxyl toluene) provided quantity 7.5g/2.5kg feed.
[2]C.M.C (carboxy methyl cellulose) provided quantity 25g/2.5 kg feed.
[3]Composition of vitamin mineral mix (PREMIX PLUS) (quantity/2.5kg) Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride,150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L- lysine, 10 g; DL- Methionine, 10 g; Selenium, 50 ppm; Satwari, 2500 mg;
[4]Composition of HUFA (EPA:DHA, 1:2)-Triomega omega-3 fatty acid capsule (GLETEC INS., USA).
[5]DE (digestible energy) calculated (K cal/ 100g) = (% Crude X protein 4) + (% Crude X fat 9) + (% TC X 4)
[6]P/E ratio (protein/energy ratio)
Control T_0H_0 (basal feed+117 mg/kcal, P/E ratio); T_1H_0 (basal feed +100 mg/kcal, P/E ratio); T_2H_0 (basal feed +133 mg/kcal, P/E ratio); T_3H_1 (basal feed +100 mg/kcal, P/E ratio+1% HUFA) and T_4H_1 (basal feed + 133 mg/kcal, P/E ratio+1% HUFA)

A P/E ratio of 117 mg/kcal was selected for the control group (T0H0) based on the findings in channel catfish fingerlings (Magnalik 1986). Fish meal and soybean, were used as protein sources; sunflower oil was used as the lipid source; corn, rice powder, and wheat flour were used as carbohydrate sources. All ingredients were thoroughly mixed with water to make dough. The dough was steam cooked for 10 min in a pressure cooker. Vitamin-mineral premix was mixed after cooling and 1% HUFA (GLETEC INS., USA) was added in T3 and T4 diets. Finally, the dough was pressed through a hand pelletizer to produce uniform size pellets and then sun dried for 4 h. The pellets were then kept in a hot air oven (50-60°C) overnight for complete drying, packed in polythene bags, and stored at 4°C throughout the experimental period. Feed was given to satiation level for 60 days twice daily. The daily ration was divided into two parts: 2/3rd of the total ration given at 09:00 h and 1/3rd at 18:00 h. The uneaten feed and fecal matter were removed by siphoning out about 50% of the tank water on alternate days.

Proximate analysis. The proximate composition of the experimental diets and fish muscle was determined following the standard methods of AOAC (1995) (Table 1). The moisture content was determined by drying at 105^0C to a constant weight. Nitrogen content was estimated by automated Kjeldahl apparatus (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) and Crude protein (CP) was estimated by multiplying nitrogen percentage by 6.25. Ether extract (EE) was measured using a Soxtec system (1045 Soxtec extraction unit, Tecator, Sweden) using diethyl ether (boiling point, 40-60^0C) as a solvent and ash content was determined by incinerating the samples in a muffle furnace at 600^0C for 6 h. Total carbohydrate was calculated thus:

total carbohydrate % = 100-CP%+EE%+Ash%). The digestible energy (DE) value of experimental diets and tissue was calculated as described by Halver (1976). The protein energy ratio of different experimental diets was calculated by varying protein percentage and maintaining the constant digestible energy values in different diets (Table 1).

Growth study. Fish were weighed at the start and at 15 day intervals until termination of the experiment on the 60th day. The growth performance of *P. hypophthalmus* fingerlings was evaluated in terms of weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and feed conversion efficiency (FCE) using the following equations:

Weight gain (%) = (final weight-initial weight) / initial weight x 100;

SGR = 100 x (log average final weight-log average initial weight)/number of culture days;

FCR = Total feed given (dry weight) (g) / weight gain (wet weight) (g);

PER = Total wet weight gain (g)/crude protein fed (g);

FCE=Weight gain (wet weight) (g)/ total feed given (dry weight) (g)

Tissue homogenate preparation. At the end of the feeding trial, three fish per treatment were sampled. The fish were anaesthetized with clove oil at 50 μl^{-1}, and dissected to collect the whole intestine (without their content) for amylase and protease assays. A 5% homogenate was prepared with chilled sucrose solution (0.25 M) in a glass tube using a mechanical tissue homogenizer. The tube was kept in ice to avoid heating. The homogenate was centrifuged at 5000 x g for 10 min at 4°C in a cooling centrifuge. The supernatant was collected and stored at -20°C for enzyme studies

Enzyme assays

Amylase assay. Amylase activity was measured by estimating the reduced sugars produced by the action of gluco-amylase and alpha-amylase on carbohydrates using di-nitro-salicylic acid (DNS) as described by Rick and Stegbauer (1974). The reaction mixture consisted of 1 % (w/v) starch solution, phosphate buffer and tissue homogenate. The reaction mixtures were incubated at 37^0C for 30 min followed by addition of DNS, and kept in boiling water for 5 min. After cooling, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard, and amylase activity was expressed as millimoles of maltose released from starch per minute at that temperature.

Protease assay. Protease activity was determined as described by Drapeau (1974). The reaction mixture consisted of 1% (w/v) casein in 0.05M Tris-PO4 buffer (pH-7.8) and was incubated for 5 min at 37^0C. Then the tissue homogenate was added. Ten minutes later, the reaction was stopped by the addition of 10% trichloroacetic acid (TCA), followed by the filtration of the samples. The reagent blank was made by adding tissue homogenate just before stopping the reaction with TCA and with no incubation. One unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A280 per minute at 37^0C and pH 7.8.

Protein estimation. Protein estimation in the intestinal tissue was carried out using alkaline $CuSO_4$ and freshly prepared 1 N Folin-Ciocalteau reagents (Lowry et al. 1951).

Texture measurement. Texture profile analysis (TPA) was performed at ambient temperature with a texture analyzer (TA-XT2, Stable Micro Systems, UK) equipped with a 5 kg load cell. Fillet muscle of uniform thickness from different treatments groups were prepared (25 mm thickness) and were placed on the centre portion of the platform of the testing machine. A five mm diameter spherical stainless steel probe was used for the study. Analysis was done with a pre-test speed of 0.5 mm/sec, test speed of 0.2 mm/sec and a post-test speed of 5 mm/sec. The penetration distance of the probe was kept at 1 mm and the time duration was adjusted to 5 sec. A force curve time was recorded from where peak force and area was calculated.

Fatty acid profile of the muscle. Fatty acids profile analysis, and extraction of lipid from the muscle was done by Folch method (Folch et al., 1957). The extracted lipid was subjected to fatty acids methyl esters (FAME) preparation and analyzed in GC-MS for the fatty acid profile. The AOAC (1995) method was followed to esterify the lipid extract. FAME was prepared from the lipids extracted from samples by heating with the

methanolic NaOH first and then with BF3- methanol for esterification. 5 ml n–heptane was added to recover the methyl esters in the organic phase. Saturated NaCl solution was added to the mixture and the aqueous and organic layers were separated using a separating funnel. The upper n-heptane phase was pipetted out and were kept refrigerated in 10 ml glass vials until further analysis.

Fatty acids were separated using a Shimadzu Qp 2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) equipped with a carbowax (30 m × 0.25 mm ID; 0.25µm film thickness) capillary column (Cromlab S.A). Helium was used as the carrier gas. Injector and detector temperatures were set at 2500C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 500C for 2 min and then increased at a rate of 100C per min to a final temperature of 2300C. FAME esters were separated at constant pressure (23.1 kPa) and peaks were identified by comparing the mass spectra with the mass spectral data base.

Statistical Analysis. Mean values of all parameters were subjected to one way ANOVA to study the treatment effect and Duncan's Multiple Range Tests (DMRT) were performed to determine the significant differences between the mean values. Comparisons were made at the 5% probability level.

Results

Proximate composition of fish muscle. Significantly higher (P<0.05) protein deposition was found in T2H0 and T4H1 groups fed with 133 mg/kcal (P/E ratio) diet. Similarly, lower (P<0.05) fat deposition in fish muscle was observed in the T2H0 and T4H1 groups. Trend for ash content was similar to that of crude protein and was higher in the T2H0 and T4H1 groups. Moisture content was significantly higher (P<0.05) in the T4H1 group (Table 2).

Table 2. Proximate composition of the fish muscle of *P. hypophthalmus* fingerling fed with varying P/E ratio and HUFA supplemented diet after 60 days. (%DM basis ±SE)

Treatmt	*Moisture*	*Crude protein*	*Crude fat*	*Ash*	*TC*	*Digestible energy**
T_0H_0	80.82^{c}±0.20	65.56^{c}±0.19	14.94^{a}±0.08	9.29^{c}±0.08	10.19^{c}±0.20	437^{b}±0.08
T_1H_0	71.62^{a}±0.25	64.88^{a}±0.06	16.64^{c}±0.15	8.84^{a}±0.08	9.62^{b}±0.17	447^{c}±0.42
T_2H_0	78.82^{b}±0.50	66.52^{d}±0.16	15.08^{b}±0.03	10.29^{d}±0.15	8.09^{a}±0.17	434^{a}±0.46
T_3 H_1	71.47^{a}±0.23	65.41^{b}±0.19	16.61^{c}±0.12	9.00^{b}±0.05	8.96^{a}±0.34	447^{c}±0.63
T_4 H_1	81.33^{c}±0.11	67.10^{d}±0.12	15.28^{b}±0.13	10.27^{d}±0.13	7.33^{a}±0.34	435^{b}±0.61

Mean values in the same column with different superscript differ significantly (P<0.05).
DM-Dry matter
Calculated Digestible Energy* (K cal/ 100g) = (% Crude X protein 4) + (% Crude X fat 9) + (% TC X 4)
TC-Total Carbohydrate. Data expressed as Mean ± SE, (n=3)

*Growth parameters. G*rowth parameters of fish in the different experimental groups at the end of feeding trials are shown in Table 3. Supplementation of different P/E ratio and HUFA diet significantly affected weight gain % and specific growth rate (SGR) of the experimental groups. Significantly higher (P<0.05) weight gain % and SGR were recorded in the T4H1 group fed 133 mg/kcal, P/E ratio with 1% HUFA supplemented diet and was comparable to T2H0 group. Lowest weight gain % value and SGR were observed in T1H0 group fed a non HUFA supplemented diet having P/E ratio of 100mg/kcal and was similar to T3H1. Similarly, higher (P<0.05) FCE was recorded in the T4H1 group fed a diet having P/E ratio of 133 mg/kcal with 1% HUFA supplementation, similar to the T2H0 group fed a diet with similar P/E ratio but not supplemented with HUFA. Highest (P<0.05) FCR value was recorded in the T1H0 group containing P/E ratio of 100 mg/kcal and was significantly different from all other treatment groups. Lowest value of FCR was noticed in T4H1 group fed the diet having P/E ratio of 133 mg/kcal with 1% HUFA incorporation and was similar to T2H0 group. Lower PER value was exhibited by T4H1 group and was similar to T2H0 and control group T0H0, while higher PER value was observed in T3H1 and did not vary significantly (P>0.05) from T1H0 group (Table 3).

Table 3. Growth parameters of *P. hypophthalmus* fingerlings fed with varying P/E ratio with HUFA supplemented diet for 60 days.

Treatmts	*P/E ratio*	*% Body wt gain*	*SGR*[1]	*FCR*[2]	*FCE*[3]	*PER*[4]
T_0H_0	117 mg/kcal	134.76^{b}±2.47	0.95^{b}±0.01	3.38^{b}±0.05	0.30^{b}±0.01	0.86^{a}±0.01
T_1H_0	100 mg/kcal	114.85^{a}±4.59	0.85^{a}±0.02	3.60^{d}±0.10	0.28^{ab}±0.0	0.94^{b}±0.03
T_2H_0	133 mg/kcal	152.57^{c}±4.18	1.03^{c}±0.02	2.95^{a}±0.07	0.34^{c} ±0.01	0.85^{a} ±0.02
T_3 H_1	100 mg/kcal	119.41^{a}±5.09	0.87^{a}±0.03	3.49^{c}±0.15	0.29^{a} ±0.03	0.96^{b}±0.04
T_4 H_1	133 mg/kcal	159.04^{c}±5.03	1.06^{c}±0.02	2.94^{a}±0.08	0.34^{c} ±0.02	0.84^{a} ±0.02

Mean values in the same column with different superscripts differ significantly (P<0.05).
[1]SGR (specific growth rate).
[2]FCR (feed conversion ratio).
[3]FCE (feed conversion efficiency). [4]PER (protein efficiency ratio))
Data expressed as Mean ± SE, (n=3)

Amylase and protease enzyme assays. Incorporation of diets with different P/E ratios and HUFA significantly affected intestinal amylase and protease activity in *P. hypophthalmus* fingerlings. Fish fed with T2H0 and T4H1 diets containing higher P/E ratio (133 mg/kcal) had significantly higher (P<0.05) protease activity in the intestine. Significantly lower (P<0.05) protease activity was recorded in the T1H0 and T3H1 group. Higher amylase activity was observed in T1H0 group which was fed a lower P/E ratio diet. T2H0 and T4H1 groups showed lower amylase activity

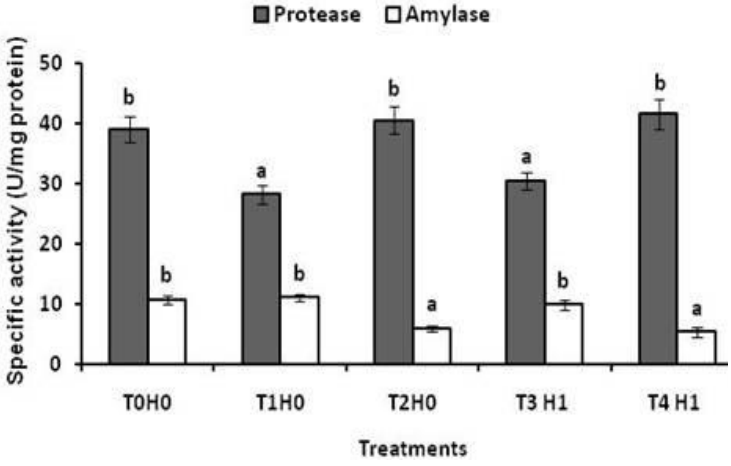

Fig.1. Protease and amylase activity in the intestine of *P. hypophthalmus* fingerlings fed with varying P/E ratio and HUFA supplemented diet at the end of 60 days. Different superscripts (a, b, c) in the same series differ significantly (P<0.05). Data expressed as Mean ± SE (n=3).

Control T_0H_0 (basal feed+117 mg/kcal, P/E ratio); T_1H_0 (basal feed +100 mg/kcal, P/E ratio); T_2H_0 (basal feed +133 mg/kcal, P/E ratio); T_3H_1 (basal feed +100 mg/kcal, P/E ratio+1% HUFA) and T_4H_1 (basal feed + 133 mg/kcal, P/E ratio+1% HUFA)

Fish muscle texture. Effects of different P/E ratio with HUFA containing diets on the muscle texture are presented in Table 4. Lowest muscle hardness value was observed in the T4H1 and T3H1 group fed with 1% HUFA supplemented diet and highest was observed in the non-HUFA fed T1H0 group. Adhesiveness, springiness, gumminess, and chewiness values were significantly (P<0.05) higher in T3H1 and T4H1 groups fed a HUFA incorporated diet. (Table 4).

Table 4. Muscle texture of the *P. hypophthalmus* fingerlings fed with varying P/E ratio and HUFA supplemented diet at the end of 60 days.

Treatments	*Hardness(g)*	*Adhesiveness (gs)*	*Springiness(S)*	*Gumminess (g)*	*Chewiness (g)*
T_0H_0	135.39^{d}±0.37	-25.03^{c}±0.20	0.82^{b}±0.005	65.61^{b}±0.72	53.59^{b}±0.80
T_1H_0	167.42^{e}±0.39	-17.07^{d}±0.35	0.73^{a}±0.005	61.19^{a}±0.77	44.66^{a}±0.52
T_2H_0	113.78^{b}±0.33	-11.70^{e}±0.44	0.87^{c}±0.005	71.11^{c}±0.74	62.22^{c}±0.25
T_3 H_1	117.30^{c}±0.39	-32.89^{b}±0.29	0.91^{d}±0.005	87.41^{d}±0.60	79.54^{d}±0.54
T_4 H_1	106.07^{a}±0.46	-38.27^{a}±0.36	0.94^{e}±0.005	89.37^{d}±0.51	87.75^{e}±0.63

Mean values in the same column with different superscript differ significantly (P<0.05).
Data expressed as Mean ± SE, (n=3)

Fatty acid composition of fish muscle. Fatty acid profiles of experimental diets and fish muscle are presented in Table 5 and Table 6, respectively. Higher EPA and DHA content were observed in T3H1 and T4H1 groups diets. Muscle fatty acid analysis revealed significantly (P<0.05) higher EPA and DHA deposition in fish muscle of T4H1 groups fed with 1% HUFA supplementation. Similarly, T4H1 group also exhibited

significantly (P<0.05) higher total unsaturated fatty acid and total ω-3 fatty acid deposition.

Table 5. Fatty acids profile of the different experimental diets.

Fatty acids (% of total fatty acids)	*Treatments*				
	T_0H_0	T_1H_0	T_2H_0	T_3 H_1	T_4 H_1
C14:0	3.62±0.01	3.21±0.01	1.89±0.01	4.24±0.02	4.40±0.01
C15:0	0.36±0. 02	0.35±0.01	0.42±0.01	0.52±0.01	0.37±0.03
C16:0	18.72±0.01	18.44±0.03	14.95±0.03	12.37±0.02	14.98±0.03
C16:1,ω-7	2.40±0. 02	2.63±0.01	2.63±0.02	4.29±0.01	5.52±0.01
C17:0	0.61±0.01	0.68±0.02	0.79±0.01	0.71±0.02	0.85±0.02
C18:0	5.38±0.01	0.08±0.01	0.32±0.03	0.93±0.01	0.94±0.01
C18:1, ω-9	17.17±0.02	14.56±0.03	28.09±0.02	23.17±0.03	25.00±0.01
C18:2, ω-6	29.35±0.01	30.71±0.01	24.70±0.03	18.80±0.01	18.29±0.01
C18:3, ω-3	1.35±0.01	4.27±0.02	4.67±0.01	7.57±0.01	6.94±0.03
C20:0	1.04±0.02	1.63±0.01	1.51±0.01	1.29±0.02	1.13±0.01
C20:1, ω-9	1.68±0.02	2.26±0.03	3.08±0.03	2.37±0.01	1.88±0.02
C20:2, ω-7	0.25±0.01	0.31±0.01	0.32±0.02	0.34±0.01	0.31±0.01
C20:3, ω-7	0.11±0.01	0.13±0.02	0.05±0.01	0.24±0.03	0.26±0.02
C20:4, ω-6	0.77±0.02	0.68±0.01	0.97±0.01	0.99±0.01	1.68±0.01
C20:3, ω-3	0.10±0.01	0.08±0.02	0.11±0.03	0.49±0.01	0.70±0.02
C22:1, ω-9	11.99±0.01	14.39±0.01	7.97±0.03	7.39±0.01	6.48±0.02
EPA (C20:5, ω-3)	1.51±0.02	2.98±0.01	3.12±0.01	6.79±0.03	7.03±0.01
DHA(C22:6, ω-3)	3.60±0.02	2.61±0.01	4.43±0.01	7.49±0.01	7.92±0.01
Σ ω-3	6.56±0.01	9.94±0.01	12.33±0.02	22.34±0.03	22.59±0.03
Σ ω-6	30.12±0.01	31.39±0.01	25.67±0.01	19.79±0.02	19.97±0.02
ω-3/ ω-6 ratio	0.21±0.02	0.31±0.01	0.48±0.02	1.12±0.01	1.13±0.02

Data expressed as Mean ± SE (n=3). Σ - indicate sum total

Table 6. Fatty acids composition (as % of total fatty acids) of *P. hypophthalmus* fingerlings fed with varying P/E ratio and HUFA supplemented diet for 60 days.

Fatty acids (%)	*Experimental groups*				
	T_0H_0	T_1H_0	T_2H_0	T_3 H_1	T_4 H_1
C14:0	8.11^{e}±0.01	5.78^{b}±0.02	3.33^{a}±0.02	6.60^{d}±0.03	6.40^{c}±0.03
C15:0	0.45^{ab}±0.01	0.44^{a}±0.01	0.57^{c}±0.01	0.73^{d}±0.01	0.46^{b}±0.01
C16:0	32.71^{c}±0.02	35.22^{e}±0.01	29.91^{b}±0.02	34.16^{d}±0.02	27.84^{a}±0.01
C16:1,ω-7	0.58^{c}±0.01	0.35^{b}±0.01	0.18^{a}±0.02	0.58^{c}±0.01	1.08^{d}±0.01
C17:0	0.36^{a}±0.02	0.52^{c}±0.01	0.75^{d}±0.02	0.82^{e}±0.02	0.47^{b}±0.01
C18:0	5.78^{b}±0.01	8.91^{e}±0.01	7.47^{c}±0.01	8.32^{d}±0.01	4.76^{a}±0.01
C18:1, ω-9	22.72^{d}±0.01	22.07^{b}±0.01	22.66^{c}±0.01	23.18^{e}±0.01	22.02^{a}±0.01
C18:2, ω-6	18.38^{c}±0.02	16.35^{b}±0.01	22.51^{d}±0.02	15.04^{a}±0.02	24.33^{e}±0.01
C18:3, ω-3	1.35^{c}±0.01	1.01^{a}±0.01	1.57^{d}±0.01	1.05^{b}±0.05	2.04^{e}±0.02
C20:0	0.28^{c}±0.01	0.28^{c}±0.01	0.23^{a}±0.02	0.35^{d}±0.01	0.25^{b}±0.01
C20:1, ω-9	1.26^{e}±0.02	0.85^{a}±0.01	1.14^{b}±0.02	1.22^{d}±0.01	1.18^{c}±0.01
C20:2 ω-7	0.75^{a}±0.01	0.82^{b}±0.02	1.02^{e}±0.01	0.86^{c}±0.05	0.88^{d}±0.02
C20:3, ω-7	1.35^{c}±0.02	1.54^{e}±0.02	1.52^{d}±0.01	1.12^{b}±0.01	1.02^{a}±0.02
C20:4, ω-6	2.03^{c}±0.01	2.87^{d}±0.02	2.87^{d}±0.01	1.78^{b}±0.01	1.48^{a}±0.02
C20:3, ω-3	0.12^{a}±0.01	0.12^{a}±0.02	0.20^{c}±0.01	0.17^{b}±0.01	0.21^{c}±0.02
C22:1, ω-9	0.82^{e}±0.02	0.23^{a}±0.01	0.48^{b}±0.02	0.62^{c}±0.01	0.65^{d}±0.02
EPA (C20:5, ω-3)	0.52^{b} ±0.01	0.32^{a} ±0.02	0.64^{c}±0.01	0.92^{d}±0.01	1.58^{e}±0.01
DHA (C22:6, ω-3)	2.42^{b} ±0.02	2.02^{a} ±0.01	2.98^{d}±0.01	2.48^{c}±0.01	3.35^{e}±0.01
Σ Saturated	47.68^{c}±0.01	51.15^{e}±0.02	42.26^{b}±0.02	50.98^{d}±0.02	40.17^{a}±0.01
Σ Unsaturated	52.30^{c}±0.02	48.55^{a}±0.01	57.76^{d}±0.02	49.02^{b}±0.02	59.82^{e}±0.01
Saturated/Unsaturated	0.92^{c} ±0.01	1.05^{e} ±0.01	0.73^{b}±0.01	1.03^{d}±0.01	0.67^{a}±0.02
Σ ω-3	4.42^{b} ±0.01	3.47^{a} ±0.02	5.38^{d}±0.01	4.62^{c}±0.02	7.18^{e} ±0.02
Σ ω-6	20.42^{c}±0.01	19.22^{b} 0.01	25.38^{e}±0.02	16.82^{a}±0.01	25.82^{e}±0.01
ω-3/ ω-6 ratio	0.22^{b}±0.02	0.17^{a} ±0.02	0.22^{b}±0.02	0.27^{c}±0.02	0.28^{c}±0.02

Mean values in the same row with different superscripts (a, b, c, d, e) differ significantly (P<0.05). Data expressed as Mean ± SE (n=3). Σ - indicate sum total

Discussion

The primary aim of the experiment was to delineate the effect of different (P/E) ratio with 1% HUFA supplemented diet on growth and flesh quality of *P. hypophthalmus.*

Higher WG% and SGR of T4H1 and T2H0 groups might be attributed to the higher protein and P/E ratio content in their diets utilized for growth. Our results are in agreement with the findings that high protein levels and high P/E ratios produce significantly higher WG% and SGR in rabbit fish (*Siganus canaliculatus*) fry (Yousif et al. 1996). Similarly, higher SGR has been observed in Nile tilapia as diet protein level increased from 25-45% (Hafedh-Al, 1999) and SGR and WG increased as dietary protein levels increased from 7.3 to 44.24% in the Nile tilapia (Ogunji and Wirth, 2000).

T4H1 and T2H0 groups showed significantly higher FCE which may be due to efficient utilization of higher P/E ratio and protein content diet. Our findings agree with findings that better FCE has been observed with high protein and high P/E ratio supplemented diet in *S. canaliculatus* fry (Yousif et al., 1996). Significantly higher FCR of T1H0 might be attributed to lower P/E ratio diet where deficiency of adequate protein to support body growth might have hampered fish growth. At lower P/E ratio, proteins are primarily utilized for energy purpose rather than growth. With higher P/E ratio in T2H0 and T4H1 groups sufficient protein might have been utilized for growth hence exhibiting significantly lower FCR. Our findings are corroborated by other results indicating that reduced FCR is associated with increased protein level in the diet of *P. Hypophthalmus* (Liu et al., 2011).

Lower observed PER might be attributed to the higher protein containing diet fed to T4H1 and T2H0 groups. Higher PER observed in the T3H1 group may be due to low dietary protein which might have been utilized efficiently by *P. hypophthalmus* fingerlings for protein synthesis. Similarly higher PER were achieved in Asian seabass (*Lates calcarifer*) fed diets with 35% protein than 50% protein (Catacutan et al., 1995).

Significantly higher protein deposition in the T2H0 and T4H1 groups might be due to higher protein content in the diets, as the deposition of nutrients in the fish body depends on the nutrient content of the diets. Similar results were obtained in red tilapia (Santiago and Laron, 1991) but not in blue tilapia (Winfree and Stickney, 1981). The fish in groups T2H0 and T4H1 showed significantly lower lipid deposition which may be due to higher P/E ratio diet. Our results are consistent with the reports on other fish (El-Sayed and Teshima, 1992). Significantly higher ash content in the T2H0 and T4H1 groups might be due to higher quantity of fish meal incorporated in their respective diets to achieve a higher P/E ratio. Protein is hydrophilic in nature and it has the capacity to bind to water; this may be the reason for significantly higher moisture content in the T4H1 group.

Dietary protein level generally influences protease activity in fish (Gangadhara *et al.,* 1997). Higher protease activity recorded in the T2H0 and T4H1 groups might be due to higher P/E ratio (133 mg/kcal) incorporated in the diets of respective groups as activity of amylase depends on the content of carbohydrate in the diet. Higher amylase activity may be attributed to lower P/E ratio and high carbohydrate content in the diet of T1H0 group. T2H0 and T4H1 groups were supplemented with lower carbohydrate therefore amylase activity was reduced. Furthermore, variations in digestive enzymes activities may be related to the structure of protein, energy metabolism, and duration of retention of feed in the digestive tract which in turn depends on crude fibre and physical consistency of the diet (Venkatesh et al., 1986).

Various researches have reported consumers' preferences for wild caught fish than for farmed fish due to superior organoleptic qualities and firmer texture. Texture is a complex trait involving sensory attributes of firmness, chewiness, dryness, moistness, and mouth feel (Haard, 1992). High quality traits of texture involve high fillet firmness and low lipid contents. Farmed fish having more than 18% fat content, resulting from very high energy diets, may have a detrimental effect on texture and processing characteristics. In the present study, all the treatment groups exhibited normal ranges of crude fat content (14.94-16.64). The significantly higher hardness value reported in T1H0 group might be due to lower protein content. This is similar to findings reporting increasing hardness of the flesh in dentex (*Dentex dentex*) with consequent decrease in

dietary protein content (Suarez et al. 2009). Significantly lower hardness value of the present investigation may be attributed to supplementation of 1% HUFA in the diets of T3H1 and T4H1 groups. Softer texture has been found in rainbow trout fed low dietary lipid diets than in fish fed diets rich in lipids (Andersen et al., 1997). T3H1 and T4H1 groups displayed significantly higher adhesiveness, springiness, gumminess, and chewiness values. Our results are corroborated with findings that supplementation of 1% linolenic acid in European catfish (*Silurus glanis*) diet has beneficial effects on meat quality (Bogut et al., 2002).

Fatty acid composition of fish muscle is rather complex. The fatty acid composition of fish fillets reflects fatty acid composition of diets. Incorporation of fatty acid into tissues is influenced by various metabolic processes such as preferential incorporation, beta-oxidation, fatty acid elongation, and desaturation process (Kiessling, 1993). Addition of 1% HUFA in the diet of T4H1 group resulted in increased total unsaturated fatty acids deposition in the muscle which may be due to the desaturation of saturated fatty acids. Similar results were obtained by Tidwell and Robinette (1990), in channel catfish. Significantly higher ($P<0.05$) deposition of EPA and DHA in T3H1 and T4H1 might be due to the incorporation of 1% HUFA in the diet. Similarly, higher ω-3 fatty acids of T4H1 group supplemented with 1% HUFA is in agreement with results in *S. glanis* (Bogut et al., 2002).

In conclusion results of our study suggest enhanced growth and better flesh quality *in P. hypophthalmus* fingerlings could be achieved at P/E ratio 133 mg/kcal with additional supplementation of 1% HUFA in the diet. This study was conducted with fingerlings; further study with larger fish is necessary to validate the results found where flesh quality parameters may be more significant.

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