



Optimum contribution of dietary protein: energy ratio in the grey mullet (*Mugil cephalus*, linnaeus, 1758) diets

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ABSTRACT

The effect of varying dietary protein and energy levels on the growth performance, feed efficiency and tissue chemical composition by increasing dietary energy sources (lipids and carbohydrates) for flathead grey mullet, *Mugil cephalus* fingerlings were evaluated. Twelve experimental diets were formulated by four different dietary protein levels (25, 30, 35 and 40) combined with three different gross energy levels (16, 17 and 18MJ/kg diet) to provide 12 different dietary protein: energy ratios (15.43, 14.40, 13.64, 18.60, 17.46, 16.42, 21.63, 20.73, 19.20, 24.26, 23.02 and 21.79 MJ/kg diet). The present results showed that, the highest final body weight, weight gain, specific growth rate, feed efficiency and protein efficiency ratio values were recorded with increasing of dietary protein levels from 25 up to 35%, irrespective of dietary energy levels. However, irrespective of dietary protein levels, the increasing dietary energy levels (from 16 to 18 MJ/Kg diet) obtained slightly final body weight, weight gain, specific growth rate, feed efficiency and protein efficiency ratios. Feed conversion ratio values decreased ($P \geq 0.05$) either with increasing dietary crude protein or dietary energy levels. The best FCR value was recorded with dietary energy 18 MJ/kg and 35% protein. No statistical differences ($P > 0.05$) were observed for the effect of dietary protein energy ratios on whole body proximate analysis except for body ether extract contents. The body lipid deposition may indicate that, when dietary lipid was supplied in excess, a proportion of this lipid was deposit as fats. Concomitant increase ($P < 0.05$) of body lipid and protein retained were observed with increase dietary lipid levels, while negative effect on energy retained was recorded with increase energy levels. These results suggested that the diet contains 30% crude protein with 18MJ/kg⁻¹ gross energy enhanced the growth performance and feed efficiency of *Mugil cephalus*, had protein-sparing effect and increased the utilization of each dietary protein and energy sources.

INTRODUCTION

Grey mullet, *Mugil cephalus* is an extremely wide spread fish species. This specie is found in temperate and tropical waters throughout the world (Kanimozhi *et al.*, 2013). The majority of studies (Blaber, 1976, Minckley 1982, Romer and Mclachlan, 1986) on different species of mullet (*Mugil cephalus* and *Liza ramada*) have shown that the adult fish diet (>30 mm) consists of detritus, diatoms, sand

grains, crustaceans, algae and decomposed organic matter. The studies on juvenile mullet (fish < 30mm), which done by (De silva, 1980 and Loftus *et al.*, 1983), reported that juveniles are mainly carnivorous, eating planktons, micro-crustaceans, shrimp larvae and zooplankton. Fernandez (2014) showed that the diet of *Mugil cephalus* was composed of upwelling diatom (pelagic and benthic), dino flagellates (cosmopolitan and thermophilia), silica flagellates, tintinnids, copepoda, euphausiacea occasional organisms and unidentified organic remains.

Carbohydrate, protein, and lipid are used as energy sources by fish, but these organic compounds are not equally well suited for the promotion of growth and the optimal nutrient composition of feeds varies between fish species (Klaoudatos *et al.*, 2005). While herbivorous and omnivorous fish accept more than 25% carbohydrate in their diet, carnivorous fish have optima below 20% (Wilson, 1994). The ratio of dietary protein to energy is important for production more economical feeds and to minimize adverse environmental impacts (Kaushik and Medale, 1994). Protein is the cost lest micronutrient in any feed and its share is high in fish feed. Therefore, replacing dietary protein by carbohydrate or lipid not only reduces production cost but also nitrogen effluent from the culture system (Wu *et al.*, 2007). Commercial fish feed formulations tend to increase dietary lipid levels to improve feed utilization for the optimization of production (Caballero *et al.*, 1999). Actually in fish feed production sector it's reasonable to increase lipid content, spare protein, improve feed conversion, decrease the amount of waste production by the fish. Additionally, special attention is being given to the development of practical feeds that maximize nutrient retention and minimize nutrient loss (Tacon, 1997). Furthermore, carbohydrate, if well utilized by fish, would be more economic compared with lipid because of it's cheaper cost and better availability, although lipid constitutes an important source of non-protein energy source for fish (Kaushik *et al.*, 1989). Though, (Cowey and Walton, 1989 and Wilson, 1994) reported that, provide an adequate carbohydrate level in fish diet can reduce catabolism of protein for energy and for synthesis of glucose, which secondly reduces protein and increases nitrogen release.

From both economic and environmental point of view for aquaculture, the present study aiming to determine the effect of varying dietary protein and energy levels on the growth performance, feed efficiency and tissue chemical composition by increasing dietary energy sources (lipids and carbohydrates) for flathead grey mullet, *Mugil cephalus* fingerlings.

MATERIALS AND METHODS

Experimental diets

Twelve experimental diets were formulated by four different dietary protein levels (25, 30, 35 and 40) combined with three different dietary gross energy (16, 17 and 18 MJ/kg⁻¹ diet) to provide 12 different dietary proteins: energy ratios as: 15.43, 14.40, 13.64, 18.60, 17.64, 16.42, 21.63, 20.37, 19.20, 24.26, 23.02 and 21.79 as presented in (Table, 1). Diets were pelleted with laboratory pellet mill without steam conditions and stored at 4°C until use. The feed ingredients and experimental diets were analyzed following the procedure of (AOAC, 2006). The gross energy (MJ kg⁻¹ diet) contents of the diets and fish were calculated by using the following calorific values: 23.9, 39.8 and 17.6 kJg⁻¹ diet for protein, either extract and nitrogen free extract, respectively (NRC, 2011).

Fish experimental conditions

Twenty four rectangular fiberglass tanks (800L each) were used and supplied with saline water, which passed through two sandy filters. Physicochemical characteristics of water tanks were: salinity ($33.2 \pm 1.4 \text{ g L}^{-1}$), water temperature ($26.2 \pm 2.7^\circ \text{C}$), dissolved oxygen ($5.2 \pm 0.8 \text{ mgL}^{-1}$), pH (7.8 ± 0.1), and unionized ammonia (0.03 ± 0.01) during the experimental period, to maintain water quality within the optimum range for *Mugil cephalus* as recorded by (Abdel-Tawwab *et al.*, 2005). Grey mullet, *Mugil cephalus* (Linn) fingerlings were obtained from a local commercial farm and acclimated to the laboratory conditions for two weeks. Fish was fed daily ad-libitum on a commercial diet (30% CP). Fingerlings (mean initial weight $30.23 \pm 1.87 \text{ g}$) were randomly distributed at 40 fingerlings per tank using two replicates for each treatment. The experimental period lasted 126 days after start (1 June till 4th October, 2016). All groups of fish were fed with the experimental diet to visual satiation by hand two times a day (08.00 am and 16.00pm). Fish were weighed every 2 weeks to adjust the amount of feed consumed.

Table 1: Ingredients content and proximate composition of experimental diets.

Protein %	25			30			35			40		
Energy (MJ/Kg)	16	17	18	16	17	18	16	17	18	16	17	18
Fish meal	12.3	12.3	12.3	18.0	18.0	25.5	25.5	25.7	26.3	31.2	31.2	31.2
Meat meal	7.1	8.2	8.2	11.0	12.3	14.3	14.3	14.9	15.7	18.6	19.0	19.6
Soybean meal	16.2	15.6	16.7	17.2	17.2	15.2	15.2	15.5	15.2	15.2	17.1	17.2
Wheat bran	57.2	52.8	48.0	48.3	43.0	31.0	41.0	36.1	31.0	31.5	26.0	26.5
Fish oil	5.2	9.1	12.8	3.5	7.5	12.0	2.0	5.8	9.8	1.5	4.7	8.5
Vit.Min.Mix. ¹	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Proximate analysis (% ww basis)												
Crude protein	25.12	24.87	24.88	30.1	30.09	29.86	34.87	34.83	34.87	39.54	39.89	39.59
Either extract	8.33	12.18	15.79	7.12	11.07	14.89	6.21	9.94	13.91	6.20	9.31	13.61
Nitrogen free extract	39.6	36.80	34.16	35.0	31.07	29.21	30.26	27.42	24.38	24.92	22.32	18.70
Energy of crude protein	6.0	5.94	5.95	7.19	7.19	7.13	8.33	8.32	8.33	9.45	9.53	9.46
Energy of either extract	3.31	4.85	6.28	2.83	4.40	5.93	2.47	3.96	5.54	2.47	3.70	5.42
Energy of NFE	6.97	6.48	6.01	6.16	5.47	5.14	5.32	4.82	4.29	4.38	4.1	3.29
Total Gross energy ²	16.28	17.27	18.24	16.18	17.06	18.20	16.12	17.1	18.16	16.3	17.33	18.17
Fiber	8.93	8.50	8.03	8.41	8.94	8.48	8.84	8.36	7.87	8.23	7.78	7.34
Ash	9.56	9.57	9.45	9.97	10.02	9.90	11.38	11.32	11.33	12.80	12.73	12.65
Moisture	8.46	8.08	7.69	9.40	8.81	7.66	8.44	8.13	7.64	8.31	7.97	8.11
Energy of CP/Total GE	36.85	34.39	32.62	44.44	42.14	39.17	51.67	48.65	45.87	57.97	54.99	52.06
Energy of Either extract/Total GE	20.33	28.08	34.43	17.49	25.79	32.58	15.32	23.16	30.51	15.15	21.35	29.83
Energy of NFE/Total GE	42.81	37.52	32.95	38.07	32.06	28.4	33.0	28.19	23.62	26.87	23.66	18.11
P/E (MJ)	15.43	14.40	13.64	18.60	17.64	16.42	21.63	20.37	19.20	24.26	23.02	21.79

1-Vitamin-mineral mixture: vitamin A, 600IU, vitamin D, 120IU, vitamin E, 78,000 mg; vitamin K, 25,000mg; vitamin B1, 12,000mg; vitamin B3, 32,000mg, vitamin B6, 21,000mg, b12 vitamin, 110mg, vitamin D, 61,000mg, niacin, 210,000mg, folic acid, 400mg, biotin, 0.237mg, selenium, 0.21 g, iron, 82 g, manganese, 90 g; zinc, 70 g, copper, 15 g, potassium chloride, 4g; manganese oxide, 0.6g, sodium bicarbonate, 1.8g, Iodine, 1.2g, cobalt, 0.35g.

2-Gross energy (MJ/ kg⁻¹ diet) was calculated by using the following calorific values: 23.9, 39.8 and 17.6 kJ/g⁻¹ diet for protein, either extract and nitrogen free extract, respectively (NRC, 2011).

Proximate analysis

Before the experiment, 10 fish from the initial fish were randomly taken to determine initial body proximate composition. After the end of the experimental, fish were starved for 24h prior to sample collection. Ten fish samples from each replicate were collected to determine the final proximate composition. Analyses of diets and fish tissues composition were done by (AOAC, 2006), dry matter determined by drying samples in an oven at 105°C until constant weight; crude protein was measuring nitrogen by ($N \times 6.25$) after acid digestion (Kjeldahl method); crude lipid

was determined through petroleum ether extraction using the (Soxhlet method), ash was detected by incineration in a furnace muffle at 550°C for 16h, while nitrogen free extract (NFE) was calculated by difference. Fish were collectively weighed every 2 weeks, and the amount of diet in restricted fed groups was adjusted accordingly. Following overnight fasting, 10 fish at the beginning, and after 18 weeks were individually weight sacrificed to determine the proximate body composition including moisture, protein, ether extract and ash content following the (AOAC, 2006) methods. Total mortalities were recorded from each tank daily. Gross energy of body tissue (MJ/ Kg⁻¹ diet) was calculated by using the following calorific values: 23.9, 39.8 and 17.6 KJ/g⁻¹ diet for protein, ether extract and nitrogen free extract, respectively (NRC, 2011).

Performance induces

The performance of the experimental fish was calculated using the following equations:

Weight gain (g) = (Final body weight-Initial body weight).

Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g).

Feed efficiency (FE %) = (weight gain%)/Feed intake (dry matter).

Protein efficiency ratio (PER %) = Fish weight gain (g)/ Protein intake (g).

Specific growth rate (SGR, % day) = (Ln FBW- Ln IBW)/t ×100; where: FBW is final body weight (g); IBW is initial body weight (g); Ln= natural logarithmic; t = time in days.

Protein intake = (Feed intake (g) × Protein in the diet %).

Feed conversion efficiency (FCE %) = 100×(final body weight–initial body weight)/feed intake.

Protein retained (%) = (Protein deposition in final muscle fish – Protein deposition in initial muscle)/Protein intake×100.

Statistical Analysis

All data are presented as mean ±SD. Data were statistically analyzed by two way classification ANOVA (factorials design) using SPSS (version 16.0, 2016). Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed (Duncan, 1955), at $P \leq 0.05$ level. The relationship between growth and feed utilization indices was tested using correlation analysis.

RESULTS AND DISCUSSION

The combined effects of dietary both protein levels and energy levels on growth performance are shown in (Table, 2). With all treatments, a significant and progressive increase ($P < 0.05$) of final body weight was recorded with the increase of protein levels from 25 up to 35%, irrespective of dietary energy levels. However, irrespective of dietary protein levels increasing dietary energy levels (from 16 to 18 MJ/Kg diet) obtained slightly final body weight gain. The interaction between protein and energy levels showed a significant ($P < 0.05$) effect of on final body weight with highly correlation values ($R^2 = 0.82$, Fig.1). Considering the effect of dietary protein energy ratios on final body weight, recorded the highest final body weight ($P < 0.05$) values for fish fed 30% crude protein with 18 MJ/Kg diet. The same trend was observed either the effect of dietary energy levels irrespective dietary protein level (Fig. 2 a) or vice versa (Fig. 2b). Similar results were obtained for trout *Rainbow trout* (Sanchez-Vazquez *et al.*, 1998 and 1999).

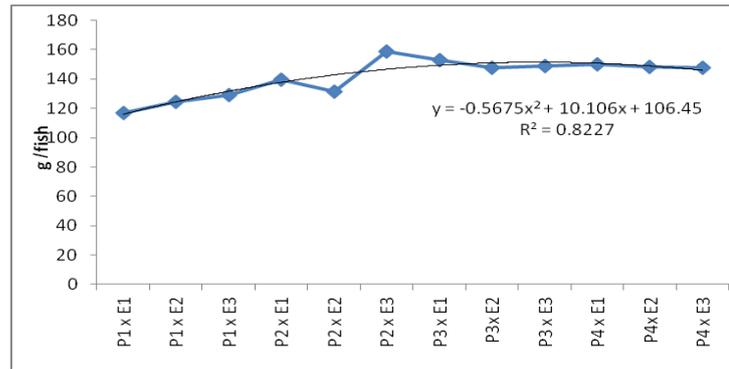


Fig. 1: The correlation between the interaction effect of dietary protein and energy levels and fish final body weight.

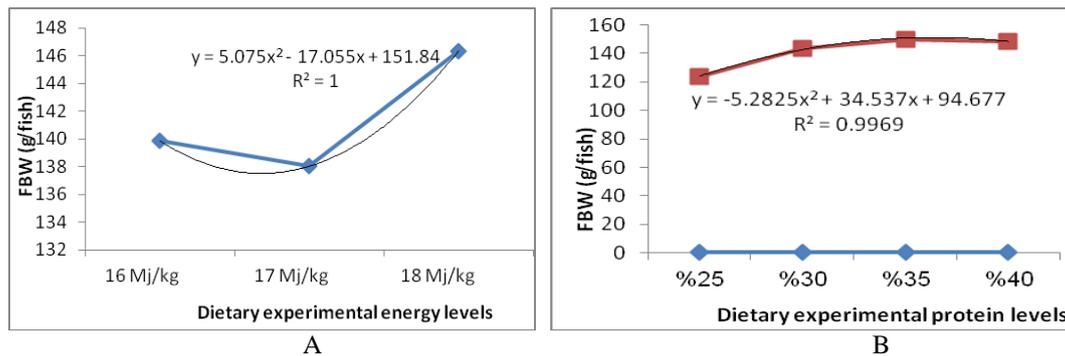


Fig. 1: The correlation between the effect of dietary protein (a) and energy levels (B) irrespective each other and fish final body weight.

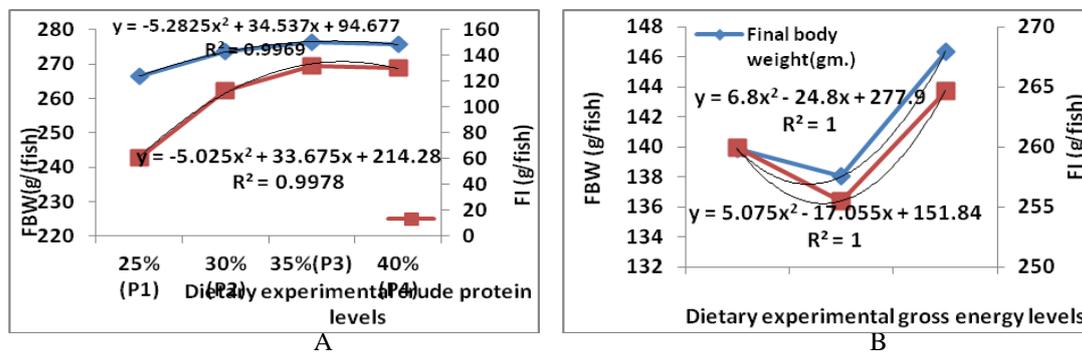


Fig. 2: The correlation between the effect of dietary protein (a) and energy levels (B) irrespective each other with fish final body weight against feed intake.

Though, the most optimal growth performance (Table, 2) in this experiment resulted in both treats contained (30% crude protein vs. 18KJ g⁻¹ diet and 35% crude protein vs. 16 KJ g⁻¹) compare with the other treatments. While, growth performance was significantly reduced (P<0.05) in the present experiment when the protein content of the diet was lower than 30% irrespective of dietary energy level. The mentioned effect, seemed to be related to the high carbohydrate levels in 25% diet (Table, 1), which led to decreased growth performance as reported by (Helland and Helland,1997). In the same manner, previous researches recorded that carbohydrate utilization differently among species (Wilson, 1994, Hemer *et al.*, 2002 and Krogdahl *et al.*,2005). Where, herbivorous or omnivorous fish species, such as grass carp, *Ctenophoryngodon idella* (Lin, 1991) and Nile tilapia, *Oreochromis niloticus* × *O. aures* (Shiau and Peng, 1993), showed better metabolic synthesis of carbohydrate than cold water. In contrast, marine carnivorous fish species, such as Atlantic salmon, *Salmo salar* (Helland *et al.*, 1991) and Yellowtail, *Seriloa quinqueradiata* (Shimeno,

1991) had a less ability to use carbohydrates in their diets. The different efficiency for carbohydrate synthesis between species, due to their natural habitat, longer digestive tract and the activity of digestive tract.

Table 2: Growth performance of mullets fingerling, *Mugil cephalus* fed the experimental diets for 18 weeks.

Dietary Crude protein (% CP)	Initial body weight (gm.)	Final body weight (gm.)	Gain weight (gm.)	Specific growth rate (%/day)	Percentage Weight Gain (%)
25 P1	30.31±2.51	123.67±6.3	93.40 ^c ±4.9	1.11 ^b ±0.16	308.15 ^c ±3.98
30 P2	30.62±2.03	143.41 ^b ±9.5	112.79 ^b ±8.5	1.22 ^a ±0.14	368.35 ^b ±4.41
35 P3	30.11±2.33	149.96 ^{ab} ±2.7	119.85 ^b ±2.3	1.28 ^a ±0.17	398.04 ^b ±22.55
40 P4	29.9±2.47	148.57 ^{ab} ±2.8	118.67 ^b ±2.6	1.27 ^a ±0.11	396.89 ^b ±12.2
Gross energy levels (KJ/g ⁻¹)					
16 E1	29.25±1.8	139.86 ^b ±13.5	110.61 ^b ±11.26	1.24 ^a ±0.13	378.15 ^b ±51.19
17 E2	30.31±2.3	138.03 ^b ±11.8	107.72 ^b ±12.13	1.20 ^a ±0.15	355.39 ^b ±47.16
18 E3	31.16±2.5	146.35 ^{ab} ±12.2	115.19 ^{ab} ±11.36	1.23 ^a ±0.16	369.67 ^b ±42.25
Protein levels (%)× Gross energy levels (KJ/g ⁻¹)					
P1 × E1	28.88±2.72	117.16 ^c ±4.2	88.28 ^c ±4.5	1.11 ^b ±0.15	305.68 ^c ±51.9
P1 × E2	30.58±2.95	124.54 ^b ±4.9	93.96 ^c ±5.3	1.11 ^b ±0.13	307.26 ^c ±47.16
P1 × E3	31.43±3.01	129.38 ^b ±7.7	97.95 ^c ±6.8	1.12 ^b ±0.10	311.64 ^c ±42.25
P2 × E1	29.52±2.41	139.82 ^b ±5.1	110.30 ^b ±5.3	1.23 ^a ±0.17	373.64 ^b ±12.5
P2 × E2	31.15±2.88	131.46 ^b ±6.9	100.31 ^b ±6.1	1.15 ^b ±0.10	322.02 ^c ±18.2
P2 × E3	31.18±3.14	158.96 ^a ±6.8	127.78 ^a ±5.7	1.29 ^a ±0.23	409.81 ^a ±15.3
P3 × E1	29.16±2.25	152.94 ^{ab} ±6.5	123.78 ^a ±6.3	1.32 ^a ±0.18	424.38 ^a ±19.2
P3 × E2	30.34±2.69	147.73 ^{ab} ±5.3	117.39 ^{ab} ±5.7	1.26 ^a ±0.12	385.78 ^b ±18.6
P3 × E3	30.77±2.40	149.20 ^{ab} ±6.3	118.43 ^{ab} ±5.4	1.25 ^a ±0.19	384.89 ^b ±13.3
P4 × E1	29.74±2.87	149.94 ^{ab} ±7.6	120.20 ^a ±6.8	1.28 ^a ±0.21	404.17 ^a ±14.9
P4 × E2	29.44±2.25	148.25 ^{ab} ±5.7	118.81 ^a ±5.3	1.28 ^a ±0.14	403.57 ^a ±18.6
P4 × E3	30.55±2.91	147.48 ^{ab} ±4.5	116.93 ^{ab} ±4.9	1.25 ^a ±0.13	382.75 ^{ab} ±15.2

Values with different superscripts letters are significantly different ($p < 0.05$).

The results presented in (Table, 3) indicated that feed intake was varies between fish fed the same amount of energy level, where it's increased with increasing dietary protein levels from 25 to 35% especially for fish fed the diets contained either 16 or 18 kJ/g energy levels. Feed conversion ratio was ranged between 2.18 and 2.67.

The current study showed that with increasing FBW the feed intake was increased either the effect of dietary energy levels irrespective dietary protein level ($R^2=1$ Fig. 2 a) or vice versa ($R^2=1$ Fig. 2b) with highly correlation values against feed intake (FBW: $R^2=0.53$, FI: $R^2=0.82$, Fig. 3). The interaction between protein and energy levels showed a significant ($P < 0.05$) effect on final body weight with moderate correlation values against feed intake (FBW: $R^2=0.53$, FI: $R^2=0.82$ Fig. 3). Those results may attribute to the increase of palatability of diets with addition a higher levels of oil as energy sources which making it more attractive to the fish especially for dietary moderate crude protein levels (30-35%).

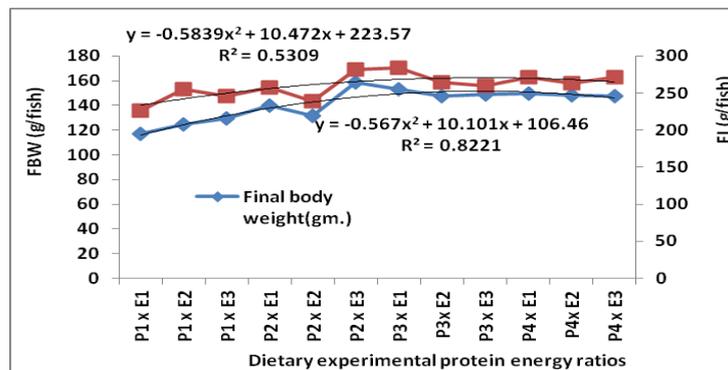


Fig. 3: The correlation between the interaction effect of dietary protein and energy levels with fish final body weight against feed intake.

A similar result was reported by (Montero *et al.*, 2005). In this connection, (Klaoudatos *et al.*, 2005) observed the higher feed intake values with lower dietary energy-high protein diet compared with the higher dietary energy-high protein for sea bream, *Sparus aurata* which indicating that regulation of the feed intake was secondarily to the dietary energy content, as observed in the present study (Figs. 3-4).

Table 3: Feed efficiency and (protein & energy) retention of fingerling, *Mugil cephalus* fed experimental diets for 18 weeks.

Treatment	Feed intake	FCR	FCE	Protein retained	Energy retained
Protein levels (%)					
25 P1	242.7 ^b ±14.6	2.60 ^b ±0.11	38.48 ^b ±1.6	24.92 ^a ±1.07	61.16 ^b ±3.11
30 P2	262.2 ^a ±15.7	2.32 ^a ±0.10	43.02 ^b ±1.8	23.14 ^a ±0.66	69.52 ^a ±2.45
35 P3	269.4 ^a ±12.7	2.25 ^a ±0.10	44.49 ^a ±1.3	19.56 ^b ±0.79	72.32 ^a ±2.46
40 P4	268.8 ^a ±4.2	2.26 ^a ±0.10	44.15 ^a ±1.3	18.10 ^b ±1.82	70.89 ^a ±3.82
Gross energy levels (KJ/g ⁻¹)					
16 E1	259.9±24.6	2.35 ^a ±0.12	42.56 ^a ±2.3	21.86 ^b ±3.2	72.06 ^a ±3.75
17 E2	255.5±11.7	2.37 ^a ±0.18	42.16 ^a ±3.5	21.00 ^b ±2.8	67.10 ^a ±3.05
18 E3	264.7±15.3	2.30 ^a ±0.13	43.52 ^a ±2.1	21.43 ^b ±3.4	66.27 ^b ±4.1
Protein levels(%)× Gross energy levels (KJ/g ⁻¹)					
25×16 P ₁ E ₁	226.8 ^c ±7.5	2.57 ^b ±0.11	38.92 ^b ±1.8	25.79 ^a ±1.62	66.95 ^b ±3.17
25×17 P ₁ E ₂	255.3 ^b ±9.7	2.72 ^b ±0.12	36.80 ^b ±1.5	23.72 ^a ±1.57	57.24 ^c ±2.80
25×18 P ₁ E ₃	246.0 ^{bc} ±11.9	2.51 ^b ±0.14	39.82 ^b ±1.7	25.25 ^a ±1.36	59.29 ^c ±3.10
30×16 P ₂ E ₁	257.5 ^b ±9.2	2.33 ^a ±0.11	42.92 ^a ±1.6	22.83 ^a ±1.43	71.53 ^a ±2.7
30×17 P ₂ E ₂	239.1 ^c ±11.0	2.38 ^a ±0.09	41.95 ^b ±1.2	22.69 ^a ±1.72	66.79 ^b ±2.15
30×18 P ₂ E ₃	281.9 ^a ±10.2	2.21 ^a ±0.10	45.33 ^a ±1.6	23.90 ^a ±1.64	70.25 ^b ±2.1
35×16 P ₃ E ₁	283.9 ^a ±11.1	2.29 ^a ±0.08	43.60 ^a ±1.5	18.66 ^b ±1.56	74.90 ^a ±3.2
35×17 P ₃ E ₂	264.0 ^{ab} ±13.4	2.25 ^a ±0.06	44.57 ^a ±1.2	20.14 ^b ±1.71	72.07 ^a ±3.6
35×18 P ₃ E ₃	259.9 ^b ±12.2	2.19 ^a ±0.10	45.57 ^a ±1.8	19.88 ^b ±1.63	70.00 ^a ±2.9
40×16 P ₄ E ₁	271.6 ^a ±10.4	2.26 ^a ±0.07	44.26 ^a ±1.9	20.15 ^b ±1.59	74.85 ^a ±2.1
40×17 P ₄ E ₂	263.3 ^{ab} ±11.8	2.22 ^a ±0.09	45.12 ^a ±1.4	17.44 ^b ±1.81	72.29 ^a ±3.0
40×18 P ₄ E ₃	270.8 ^a ±10.6	2.32 ^a ±0.10	43.18 ^a ±1.6	16.70 ^b ±1.69	62.52 ^b ±2.3

Values with different superscripts letters are significantly different ($p < 0.05$).

Table 4: Muscle body composition (ww/basis) of *Mugil cephalus* fed the experimental diets for 18 weeks.

Composition	Moisture %	Crude protein %	Ether extract %	Ash %	Gross EnergyKJ/g
Initial	68.14±1.37	16.93±0.87	11.45±0.51	3.48±0.63	26.85±1.24
Final protein levels (%).					
25 P1	67.04 ±1.16	16.34±0.83	12.63 ^b ±0.35	4.31±0.26	26.96±1.42
30 P2	66.17±1.48	16.21±0.63	13.43 ^a ±0.47	4.34±0.23	27.12±1.53
35 P3	65.61±1.37	15.63±0.70	14.15 ^a ±0.33	4.50±0.31	27.11±1.38
40 P4	65.72±1.52	16.09±0.61	13.87 ^a ±0.38	4.32±0.25	27.18±1.41
Gross energy levels (KJ/g ⁻¹).					
16 E1	66.14±1.74	16.26±0.86	13.43 ^a ±0.62	4.42±0.21	27.12±1.32
16 E2	66.05±1.88	16.11±0.71	13.34 ^a ±0.73	4.42±0.27	26.85±1.24
16 E3	66.23±1.65	15.85±0.62	13.78 ^a ±0.74	4.27±0.19	27.32±1.35
Protein levels (%)× Gross energy levels (KJ/g ⁻¹).					
25×16	66.91±1.80	16.71±0.98	12.89 ^b ±0.71	4.49±0.33	27.42±2.17
25×17	67.22±1.55	16.25±0.73	12.27 ^b ±0.49	4.26±0.41	26.60±1.95
25×18	67.00±1.62	16.07±0.86	12.74 ^b ±0.52	4.19±0.32	26.86±2.29
30×16	66.38±1.52	16.23±0.72	12.91 ^b ±0.57	4.48±0.53	26.67±2.05
30×17	65.63±2.05	16.43±0.69	13.57 ^a ±0.65	4.37±0.37	26.99±2.49
30×18	66.51±1.70	15.98±0.73	13.82 ^a ±0.71	4.18±0.49	27.69±1.98
35×16	66.11±1.68	15.31±0.76	14.11 ^a ±0.51	4.47±0.36	27.23±2.03
35×17	65.18±2.02	16.02±0.88	13.85 ^a ±0.42	4.65±0.55	26.69±1.76
35×18	65.55±1.87	15.57±0.94	14.50 ^a ±0.40	4.38±0.51	27.42±2.14
40×16	65.14±2.05	16.80±0.83	13.81 ^a ±0.44	4.25±0.36	27.14±2.05
40×17	66.16±1.89	15.72±0.74	13.73 ^a ±0.72	4.39±0.44	27.11±1.86
40×18	85.86±1.73	15.76±0.80	14.70 ^a ±0.89	4.31±0.39	27.30±2.11

Values with different superscripts letters are significantly different ($p < 0.05$).

In the present study it's clear the effect of dietary energy value for protein-sparing effect in the tested diets. So, the highest growth observed with fish fed 30% crude protein and 18 kJ/g dietary energy may regarding to increasing dietary lipid content up to (14.89%) in the above diet (Table 1). The same finding was reported by Verger *et al.* (1996) observed a protein-sparing effect by increased dietary lipid from 9 to 15% in sea bream *Sparus aurata* and from 4-16 in sole, *Solea aegyptiaca* diets (Yones *et al.*, 2018). The same trend was found by (Caballero *et al.*, 1999) for *Sparus aurata* when fish fed with 27% dietary lipid, resulted the highest fish growth compared to fish fed diets contained 22 and 15% lipid. The decreased in fish growth when fed 15% lipid, could be insufficient level to cover energy requirements and this leading to a subsequent using dietary protein for supplied energy. Moreover, (Boujard and Medale, 1994 and Woods *et al.*, 1998) explain the correlation between body weight and feed intake. They noticed that the predicts secretions from fat cells are the key signal to the brain to regulate feeding and body-fat deposition. A feedback regulatory loop with distinct steps has been hypothesized, which include a sensor monitors energy levels, hypothalamic centers that receive and integrate through leptin receptors the intensity of the signal and effector systems that influence energy intake and expenditure (Jequier and Tappy, 1999). Though, (Pitcher and Hart, 1982) showed that the ecologically important feature of digestion is the rate at which food can be processed and this determine the upper limit to intake energy and hence the upper limit of growth.

In the present study, FCR values decreased ($P \geq 0.05$) either with increasing dietary crude protein irrespective of dietary energy levels (Fig. 4, $R^2=0.99$) or dietary energy levels irrespective of dietary crude protein levels (Fig. 5, $R^2=1$). The best FCR value was obtained with dietary energy 18 Mj kg^{-1} and 30% protein, followed by higher FBW and, WG compared to those fed the other diets may be due to the enhancement effect of P/E ratio on utilization and digestibility of diet.

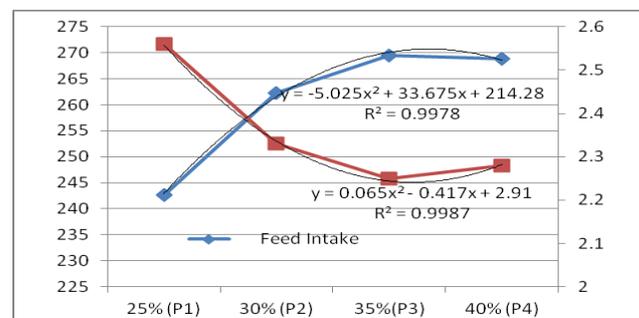


Fig. 4: The correlation between the effect of dietary protein irrespective of dietary energy levels with FCR against feed intake.

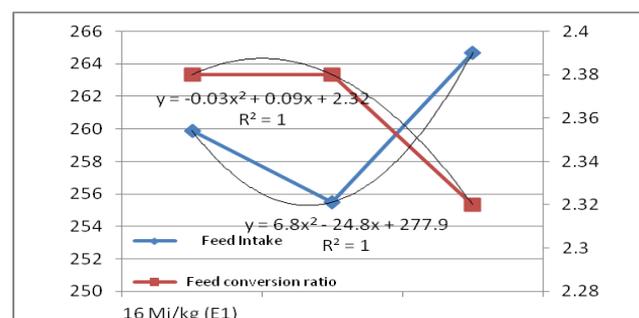


Fig. 5: The correlation between the effect of dietary energy levels irrespective of dietary protein levels with FCR against feed intake.

No statistical differences ($P > 0.05$) was observed for the influence of dietary protein energy ratios on whole body proximate analysis except for body ether extract contents (Table 4), The body lipid deposition may indicate that, when dietary lipid was supplied in excess, a proportion of this lipid was deposit as fats. The present results are agree with the results on mullet *Liza ramada* (Yones and Abdel-Tawab, 2005 and Yones *et al.*, 2016), tilapia, *Tilapia zilli*, (El-Sayed and Garling, 1988) and trout, *Salmo guirdneri* (Austreng,1979).

Concomitant increase ($P < 0.05$) of body lipid and protein retained were observed with increase dietary lipid levels (Figs. 6-7), while negative effect on energy retained was recorded with increase energy levels (Table 5), may due to the protein-sparing effect of dietary lipid (Kaushik and Medale, 1994). Data presented here showed that *Mugil cephalus* tend to less lipid efficiency than protein. Comparable results were recorded in mullet *Liza ramada* (Yones and Abdel-Tawab, 2005 and Yones *et al.*, 2016) and carp *Common carp* (Schwarz and Kirchgessner, 1995). However, the lipid deposition in fish depended to the source of energy, where its more deposit from lipid than carbohydrate sources as illustrated by (Emmans, 1994). Moreover, Lupatsch *et al.* (2003) assumed that at the highest protein-energy intake level, protein is used not just for protein deposition, but also as an energy source to deposit lipid, although at lower efficiency. The same authors showed that energy efficiency in fish would however decrease if dietary protein was used as an energy source beyond its main role for protein synthesis.

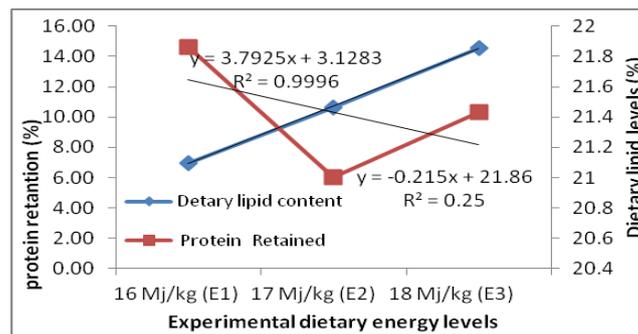


Fig. 6. The correlation between the effects of dietary energy levels irrespective of dietary protein levels on protein retained against dietary lipid levels.

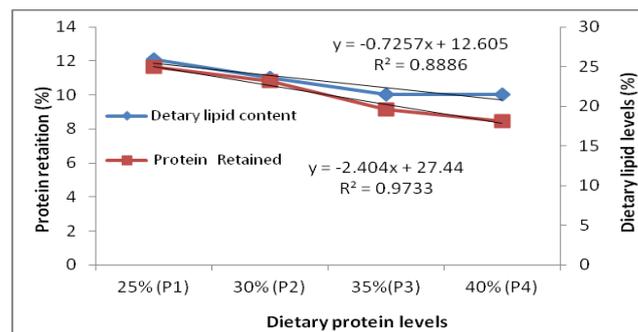


Fig. 7: The correlation between the effects of dietary protein levels irrespective of dietary energy levels on protein retained against dietary lipid levels.

Table 5: Protein and energy efficiency ratio, protein deposition and lipid deposition and retention of *Mugil cephalus* fed experimental diets for 18 weeks.

Parameters*	PER	EER	PD	LD	LR	
Protein levels (%).						
25	P1	1.54 ^a ±0.06	2.27 ^b ±0.14	15.07 ^c ±0.38	9.77 ^c ±0.13	37.06 ^b ±12.41
30	P2	1.44 ^a ±0.07	2.56 ^a ±0.14	18.05 ^b ±1.92	12.66 ^b ±2.15	47.12 ^b ±14.40
35	P3	1.28 ^b ±0.04	2.61 ^a ±0.10	18.40 ^b ±0.33	14.48 ^a ±0.77	59.47 ^b ±23.67
40	P4	1.11 ^b ±0.03	2.60 ^a ±0.19	19.34 ^b ±1.99	13.01 ^b ±1.70	56.51 ^b ±12.76
Gross energy levels (KJ/g ⁻¹).						
16	E1	1.33 ^b ±0.19	2.65 ^a ±0.15	18.17 ^b ±2.86	11.89 ^b ±2.33	66.64 ^a ±4.10
16	E2	1.32 ^b ±0.15	2.48 ^a ±0.22	17.06 ^b ±1.66	12.07 ^b ±2.05	45.39 ^b ±1.23
16	E3	1.38 ^b ±0.23	2.42 ^a ±0.15	17.92 ^b ±1.91	13.76 ^a ±2.00	35.87 ^b ±5.40
Protein levels (%)× Gross energy levels (KJ/g ⁻¹).						
25×16		1.55 ^a ±0.14	2.14 ^b ±0.13	14.69 ^c ±1.14	9.68 ^c ±0.38	25.21 ^c ±1.62
25×17		1.68 ^a ±0.09	2.16 ^b ±0.05	15.06 ^c ±0.78	9.86 ^c ±0.77	31.90 ^c ±3.70
25×18		1.60 ^a ±0.10	2.21 ^b ±0.08	15.45 ^c ±1.49	10.90 ^b ±1.13	28.06 ^c ±4.68
30×16		1.42 ^a ±0.16	2.69 ^a ±0.11	17.70 ^b ±2.13	11.66 ^b ±1.34	63.61 ^a ±0.48
30×17		1.32 ^b ±0.07	2.47 ^a ±0.14	16.33 ^c ±.54	10.79 ^b ±1.21	40.76 ^b ± 6.12
30×18		1.52 ^a ±0.11	2.52 ^a ±0.17	20.12 ^a ±2.90	15.52 ^a ±0.92	36.98 ^b ±4.74
35×16		1.24 ^b ±0.15	2.74 ^a ±0.09	18.65 ^b ±.86	15.16 ^a ±2.34	65.99 ^a ±12.88
35×17		1.28 ^b ±0.08	2.63 ^a ±0.13	18.52 ^b ±1.70	13.65 ^{ab} ±1.92	51.92 ^b ±10.84
35×18		1.31 ^b ±0.11	2.54 ^a ±0.14	18.02 ^b ±1.43	14.64 ^a ±0.83	40.50 ^b ±8.77
40×16		1.12 ^c ±0.07	2.75 ^a ±0.12	21.64 ^a ±3.24	11.07 ^b ±1.16	65.74 ^a ±12.30
40×17		1.13 ^c ±0.09	2.66 ^a ±0.16	18.32 ^b ±1.67	13.96 ^{ab} ±1.86	56.96 ^b ±11.42
40×18		1.08 ^c ±0.06	2.39 ^a ±0.14	18.07 ^b ± 2.12	13.99 ^{ab} ±1.70	37.95 ^c ±9.27

Values with different superscripts letters are significantly different ($p < 0.05$).

*PER, Protein Efficiency Ratio, EER, Energy Efficiency Ratio, PD, Protein Deposition, LD, Lipid Deposition and LR, Lipid retention.

CONCLUSION

The present results showed that, the highest final body weight, weight gain, feed efficiency (%); protein efficiency ratio (PER) and specific growth rate (SGR, % day) values were recorded with increasing of dietary protein levels from 25 up to 35%, irrespective of dietary energy levels. However, irrespective of dietary protein levels, the increasing dietary energy levels (from 16 to 18 MJkg⁻¹ diet) obtained slightly final body weight gain; weight gain, feed efficiency (%); protein efficiency ratio (PER) and specific growth rate (SGR, % day). Feed conversion ratio (FCR) values decreased ($P \geq 0.05$) either with increasing dietary crude protein irrespective of dietary energy levels or dietary energy levels irrespective of dietary crude protein levels. Concomitant increase ($P < 0.05$) of body lipid and protein retained were observed with increase dietary lipid levels, may due to the protein-sparing effect of dietary lipid suggesting that *Mugil cephalus* may tend to the synthesis of lipid is less efficient than the synthesis of protein. These results suggested the diet contains 30% crude protein with 18MJ/kg gross energy enhanced the growth performance, feed efficiency and had a sparing protein effect on mullet *Mugile cephalus*.

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ARABIC SUMMARY

النسبة المثلى لمساهمة البروتين والطاقة في علائق أسماك البورى

عبد المنعم عبد الصادق مهدى يونس^١ - أحمد كامل إبراهيم الحمادى^١ - علياء مدحت عبد الفتاح الكاشف^٢ - مدحت عبد الفتاح الكاشف^١

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تم تقييم تأثير استخدام مستويات مختلفة من البروتين والطاقة على معدلات النمو، الكفاءة الغذائية والتركيب الكيميائى بزيادة مصادر الطاقة (كربوهيدرات ودهون) فى تغذية إصبعيات أسماك البورى. حيث تم تكوين ١٢ عليقة باستخدام ٤ مستويات من البروتين مع ثلاث مستويات من الطاقة كالتالى: (٢٥-٣٠-٣٥ و ٤٠ % بروتين خام مع طاقة كلية ١٦-١٧ و ١٨ ميجا جول/كجم عليقة) للحصول على ١٢ عليقة مختلفة فلى نسبة البروتين : الطاقة كالتالى : ٤٣.١٥-٤٠.١٤-٦٤.١٣-٦٠.١٨-٤٦.١٧-٤٢.١٦-٦٣.٢١-٧٣.٢٠-٢٤.٢٠-٢٣.٠٢ ميجا جول/كجم عليقة).

اشارت الدراسة الى أن أعلى عائد لكل من الوزن النهائى، عائدالنمو، معدل النمو النوعى، الكفاءة الغذائية وكفاءة استخدام البروتين تم تسجيلها بزيادة نسبة البروتين فى العليقة من ٢٥-٣٥% بغض النظر عن مستوى الطاقة بالعليقة بينما عند غض النظر عن نسبة البروتين بالعليقة فإن زيادة مستوى الطاقة من ١٦-١٨ ميجا جول/كجم عليقة نتج عنه زيادة طفيفة فى الوزن النهائى، عائدالنمو، معدل النمو النوعى، الكفاءة الغذائية وكفاءة استخدام البروتين. إنخفضت نسبة التحويل الغذائى بزيادة نسبة البروتين بغض النظر عن كل من مستوى البروتين أو الطاقة المستخدم حيث سجلت العليقة المحتوية ٣٥% بروتين خام مع ١٨ ميجا جول/كجم عليقة افضل معامل تحويل غذائى.

لم يحدث فرق معنوى عند مستوى (٠,٠٥%) فى تركيب جسم الأسماك من المكونات المختلفة ماعدا المحتوى الدهنى للجسم سجل فروق معنوية مع العلائق المختلفة. كما اشارت الدراسة الى ان زيادة نسبة الدهن تؤدى الى زيادة نسبة تخزينه فى جسم الأسماك وعلية فإن زيادة نسبتها لها تأثير سلبى على المحتوى التخزينى للطاقة بجسم الأسماك.

اقترحت الدراسة ان استخدام العليقة المحتوية على ٣٠% بروتين خام مع طاقة كلية ١٨ ميجا جول/كجم عليقة تحسن معدلات النمو والكفاءة الغذائية لأسماك البورى علاوة على فعلها التوفيرى للبروتين و تؤدى الى زيادة الاستفادة من كل من مصادر البروتين والطاقة بالعليقة.