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Lipid and protein utilization by gilthead sea bream (*Sparus aurata L .*) under flow-through system with regard to environmental impact

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ABSTRACT

A 58 day feeding experiment was carried out to evaluate the effect of dietary protein and lipid levels on growth performance and feed utilization of gilthead sea bream fingerlings under flow-through system with regard to environmental impact. Six practical diets containing three levels of lipid (100, 150, 200 g/Kg) and two levels of protein (450, 500 g/Kg) were fed fingerlings with an average initial body weight 1.5±0.01 g. Diets were hand-distributed to duplicate groups of 60 fish, three times a day until satiation. The dietary lipid levels had significant effects on final body weight (FBW), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), energy retention (ER) and phosphorus retention (Phs R). The dietary protein levels had significant effects on FBW, FCR and PR but not on feed intake, PER, ER and phosphorus retention. The interactions of dietary protein and lipid revealed significant effects on FBW, ER and phosphorus retention but not on feed intake, FCR, PER and protein retention. Fish fed the diet containing 150 g lipid and 450 g protein per Kg diet were significantly better FBW, ER and phosphorus retention. Diet contained lipid level of 150 g/Kg and protein level of 450 g/Kg obtained satisfied growth requirements of *Sparus aurata* fingerlings to ensure minimum environmental impact and may be high economical efficiency under flow-through system.

Keywords: protein, lipid, utilization, practical, satiation, sea bream, *Sparus aurata*

INTRODUCTION

Gilthead sea bream (*Sparus aurata L.*) are the most common species cultured in land flow-through systems (Jobling, 2012). Although water is not consumed during use, flow-through systems use (but do not consume), large volumes of water per unit fish production compared to other aquaculture production systems. Effluent from flow-through aquaculture systems is characterized by large discharge volumes with low solids concentrations (True *et al*., 2004). Effluents with these characteristics are not easy to treat and implementing practical, cost effective treatment is a difficult economic and engineering challenge (Piedrahita, 2003).

The most effective way to reduce environmental impacts from flow-through aquaculture systems is to increase assimilation and retention of nutrients through manipulation of diet formulations and feeding strategies (Roque d’Orbcastel *et al*., 2008). When using nutrient-dense feeds and better feeding practices for flow-through systems, only 5% or less of feed is wasted (Sindilariu, 2007). Despite the developments in sea bream production, knowledge of the quantitative and qualitative feed requirements under flow-through system with regard to environmental impact of this species in the different stages especially nursing stage has yet to be achieved.

The improvement of fish production techniques would require a reduction of dietary protein levels in order to obtain cheaper diets and to limit the quantity of nitrogen in effluent water (Li *et al*., 2012). A crutical point for the sustainability of the aquaculture sector dependent on sustainable aqua feeds (Carter *et al*., 2012), therefore a reduction of fish meal use in fish diets, which can be partly achieved through formulation of diets of lower protein content (Sarker *et al*., 2013). The current trend in fish feed production is to increase lipid content, spare protein, improve feed conversion, decrease the amount of waste produced by the fish and special attention is being given to the development of feeds that maximize nutrient retention and minimize nutrients loss (Valente *et al*., 2011). The experiment reported here in was designed to optimize the utilization of protein and lipid in sea bream diets with regard to environmental impact under flow-through system regarding reduce protein level in the diet, maximize protein sparing effect of lipid and reduce nitrogen and phosphorus in the sea bream effluents water.

MATERIALS AND METHODS

Experimental condition

The study was carried out between 10 May and 6 July 2011 (58 days) in Fish Nutrition Lab., Shakshouk Fish Research Station (El- Fayoum)., Inland Water and Aquaculture Branch., National Institute of Oceanography and Fisheries (NIOF)., Egypt. Fish were stocked in 12 fiberglass tanks (600-l capacity) in groups of 60 fish/tank (2 replicates/diet). Tanks connected to an open flow-through system and were supplied with 1 liter min^{-1}. Each tank was equipped with individual aeration. Fish were held under natural photoperiod condition throughout the feeding trial.

Experimental fish

Sea bream fingerlings were obtained from El-Wafa Marine Fish Hatchery, Albulah-Ismailia, Egypt. Fish were healthy, free of parasites with an average body weight of 1.5±0.01 gm. Fish were randomly distributed in 12 tanks and kept for adaptation period of two weeks before the beginning of the study. Prior to weighing, 40 fish were sacrificed for determination of carcass analysis. These fish were randomly taken from all the experimental tanks. All fish were weighed in bulk every two weeks until the end of the experiment, and rate of mortality was recorded. Prior to weighing, the fish lightly anaesthetized, with a solution of clove oil with ethanol (94%) (9 parts ethanol: 1 part clove oil) and was added 2 ml of the solution into 5 L of water. After the final weighing, 20 fish from tanks were randomly taken for the determination of carcass analysis.

Experimental diets and feeding regimes

A 2 × 3 factorial design with two replicates was used in this study. Six experimental diets were formulated to contain three lipid levels (10, 15 and 20 % Dry weight) and two protein levels (50 and 45 % Dry weight). Diets were named as 10L/50P, 10L/45P, 15L/50P, 15L/45P, 20L/50P and 20L/45P (lipid/protein, % Dry weight, respectively). The experimental diets are illustrated in Table 1. All fish groups were fed three times a day (8.00, 12.00 and 17.00 h) to apparent satiation. During the acclimation period of two weeks the fish were fed a mixture of all the feeds. All diets were prepared in Fish Nutrition Lab., Faculty of Agric., Cairo University, after manually blending the dry ingredients, weighed and then mixed thoroughly with fish oil. An appropriate amount of water was added to produce stiff dough. The dough was then pelleted using a laboratory pellet machine. These diets were exposed to the air-dry for a day. After drying, the diets were manually broken to convert to crumbles size and stored in plastic bags (- 4°C) until used.

Table 1. ***Feed ingredients and composition of experimental diets (% DM basis).***

Ingredient	10L/50P	10L/45P	15L/50P	15L/45P	20L/50P	20L/45P
Fishmeal	60	55	60	55	60	55
Soybean meal	17	15	17	15	17	15
Fish oil	4	4	9	9	14	14
Starch	16	23	11	18	6	13
Premix[a]	2	2	2	2	2	2
CMC[b]	1	1	1	1	1	1
Chemical composition						
Moisture	7.80	8.27	7.57	7.16	7.21	7.67
Protein	51.10	43.90	48.11	45.31	50.18	46.50
Lipid	11.50	9.48	16.70	15.31	19.55	18.90
Ash	11.30	10.91	9.83	11.71	12.52	10.10
Total NFE [c]	26.10	35.71	25.36	27.67	17.75	24.50
Phosphorus [d]	1.49	1.36	1.49	1.36	1.49	1.36
Gross energy ($KJ\ g^{-1}$) [e]	21.11	20.31	22.35	21.54	22.63	22.69
Protein/ energy ($g\ MJ^{-1}$)	24.21	21.62	21.53	21.03	22.18	20.49

[a] Premix supplied the following minerals (g kg^{-1} of diet) and vitamins (IU or mg kg^{-1} of diet): CuSO4_5H2O, 2.0 g; FeSO4_7H2O, 25 g; ZnSO4_7H2O, 22 g; MnSO4_4H2O, 7 g; Na2SeO3, 0.04 g; KI, 0.026 g; CoCl2_6H2O, 0.1 g; Vitamin A, 900,000 IU; Vitamin D, 200,000 IU; Vitamin E, 4,500 mg; Vitamin K3, 220 mg; Vitamin B1, 320 mg; Vitamin B2, 1,090 mg; Vitamin B5, 2,000 mg; Vitamin B6, 500 mg; Vitamin B12, 1.6 mg; Vitamin C, 5,000 mg; Pantothenate, 1,000 mg; Folic acid, 165 mg; Choline, 60,000 mg.

[b] Carboxy methyl cellulose

[c] NFE (Nitrogen free extract) = 100 – (protein+ lipid + Ash).

[d] Calculated based on the standard physiological fuel values: 23 g/kg for fishmeal and 6.66 g/kg for soybean meal.

[e] Gross energy was calculated using conversion factors of 39.71, 23.41 and 17.56 and KJ g^{-1} for fat, protein, and carbohydrate, respectively, (KJ= 4.18Kcal) (Young et al., 2005).

Water quality and environmental parameters

Water temperature and pH-value were daily measured by thermometer and pH-meter device, respectively. Dissolved oxygen (DO) was measured by DO-meter and maintained above 5.0 mg l^{-1} during the feeding trial by increasing flow rate. Dissolved inorganic nutrients (NH_4, NO_3, NO_2 and PO_4) were determined by filtering water samples through Whatman GF/F glass fibre filters and freezing them immediately on dry ice. Samples were analyzed within two weeks at Chemistry Lab., Inland Water and Aquaculture Branch., National Institute of Oceanography and Fisheries (NIOF), in accordance with the methods of Clesceri *et al.* (1989) using a Spectrophotometer (model LKB Biochrom Ultrospec II.).

Chemical analysis

Diets and whole body fish were analyzed for proximate composition. Moisture was determined by oven drying at 105 ^{0}C until constant weight. Crude protein (nitrogen × 6.25) was analyzed by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Höganäs, Sweden); crude lipid by ether extraction using a Soxtec System HT (Soxtec System HT6, Tecator, Höganäs, Sweden) and ash by combustion at 550 ^{0}C for 4 h. Protein, moisture, ash and lipid were analyzed according to (AOAC, 2006). Phosphorus of whole body fish followed the same general pattern as ashing methods and was added 2 ml of HCL (conc) and 3 ml of distilled water even solubility. Using of filter paper was transferred it to empty bottle and was added distilled water until 25 ml from volume of this bottle and transferred to analyzed in Chemistry

Lab., Inland Water and Aquaculture Branch, National Institute of Oceanography and Fisheries (NIOF), in accordance with the methods of Clesceri *et al.* (1989) using a Spectrophotometer (model LKB Biochrom Ultrospec II.).

Assessment of growth and feed utilization

Growth and feed performance were described using the parameters, (all calculations based on as fed, wet basis):

Specific growth rate (SGR)(%/day) = 100 × [ln final weight (g)-ln initial weight (g)] / number of days.

Food conversion ratio (FCR) = food given (g)/increase in biomass of fish (g).

Protein efficiency ratio (PER) = increase in biomass of fish (g)/protein intake (g).

Protein retention (PR) (%) = 100 × [protein deposition (g)/protein intake (g)].

Energy retention (ER) (%) = 100 × [energy deposition (kcal)/energy intake (kcal)].

Phosphorus retention (PhsR) (%) = 100 × [phosphorus deposition (g)/phosphorus intake (g)].

Statistical analysis

Mean values are reported with a pooled standard error of a means (SEM). After confirming normality and homogeneity of variance, the data were analyzed by two-way ANOVA, using protein levels and lipid levels as the two factors (SPSS, version 17.0). Using Duncan´s multiple comparisons to compared different means. Differences were considered significant at $P < 0.05$. The relationship between FBW, PR, ER and phosphorus retention with protein or lipid intake was evaluated by applying linear regression and quadratic regression analysis as described by Sokal and Rohlf (1981).

RESULTS AND DISCUSSION

Growth performance

Summarized in Table (2) are the growth performances of experimental fish. Highly significant ($P < 0.001$) differences due to varying dietary protein and lipid levels and their interactions on final body weight (FBW) were observed. Dietary lipid levels had significant effects on FBW. Fish fed 10L or 15L diets were significantly higher FBW, being 10.64 and 10.88 g, respectively, with a little big difference. However, fish fed 20L diet recorded the least. Dietary protein levels revealed significant effects on FBW. Fish fed 50P diet were significantly higher FBW (9.86 g) compared to fish fed 45P diet (9.46 g).

Dietary protein and lipid levels interactions showed significant ($P < 0.015$) effects on FBW. Highest final body weight was recorded for fish fed 10L/50P diet (11.29 g) than fish rested. Weight gain and SGR data followed the same general pattern as FBW.

Gilthead sea bream is considered fish species required protein around 50% (Santinha *et al*., 1996). However, protein sparing effect of dietary lipid has been demonstrated (Vergara *et al*., 1996), and reducing dietary protein from 52 to 46% by increasing dietary lipid from 9 to 15% and fish grew slightly better.

The metabolic capacity of processing high dietary lipid levels is species dependent and is related to feeding habits; carnivorous fish generally being able to use higher lipid levels than omnivorous or herbivorous fish (Seiliez *et al*., 2006).

The negative effect of high lipid diets on growth has been previously reported in gilthead sea bream (*Sparus aurata*) (Company *et al*., 1999) and other species like dentex (*Dentex dentex*) (Espino´ s *et al*., 2003), sea bass (*Dicentrachus labrax*) (Pe´res and Oliva - Teles, 1999), cobia (*Rachycentron canadum*) (Chou *et al*., 2001) and meagre (*Argyrosomus regius*) (Chatzifotis *et al*., 2010). The authors suggested that the reduction in fish growth when fed high lipid diets is because of the excessive energy that reduces food consumption.

Table 2. ***Effect of different dietary protein and lipid levels and their interactions on growth performance and feed utilization parameters of gilthead sea bream (Sparus aurata L.).***

Treatment	IBW[2] (g)	FBW[3] (g)	Gain (g)	SGR (%)	FI[4]	FCR
Lipid (% DM)						
10L	1.50	10.64 a	9.14 a	3.38 a	14.97 a	1.64 b
15L	1.50	10.88 a	9.38 a	3.42 a	14.09 b	1.50 c
20L	1.50	7.46 b	5.96 b	2.76 b	11.98 c	2.01 a
SEM [1]	0.01	0.139	0.139	0.026	0.124	0.028
P ANOVA	> 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Protein (%DM)						
50P	1.50	9.86 a	8.36 a	3.22	13.64	1.66 b
45P	1.50	9.46 b	7.96 b	3.15	13.72	1.77 a
SEM	0.01	0.114	0.114	0.021	0.101	0.023
P ANOVA	> 0.05	< 0.046	< 0.046	> 0.056	> 0.575	< 0.018
Lipid × Protein (% DM)						
10L/50P	1.50	11.29 a	9.79 a	3.48 a	15.17	1.55
10L/45P	1.50	10.00 b	8.50 b	3.28 b	14.77	1.74
15L/50P	1.50	10.68 ab	9.18 ab	3.38 ab	13.85	1.51
15L/45P	1.50	11.09 a	9.59 a	3.45 a	14.33	1.49
20L/50P	1.50	7.63 c	6.13 c	2.80 c	11.90	1.94
20L/45P	1.50	7.29 c	5.79 c	2.73 c	12.07	2.08
SEM	0.01	0.197	0.197	0.036	0.176	0.040
P ANOVA	> 0.05	< 0.015	< 0.015	< 0.025	> 0.113	> 0.093

[1] ***SEM: Pooled standard error of means***
[2] ***IBW: Initial body weight***
[3] ***FBW: Final body weight***
[4] ***FI: Feed intake g/fish***

The results reported here in agreed with the obtained data by (Schuchardt *et al*., 2008) who reported that in red sea bream and red porgy, *Pagrus pagrus*, the best performances were achieved at 15% dietary lipid level, while raising dietary lipid level depressed growth. Common dentex growth was depressed at 22% dietary lipid level (Espino´ s *et al*., 2003). The range of dietary lipid levels between 9 and 17% did not seem to affect growth greatly (Espino´ s *et al*., 2003). White sea bream does not affect growth rate, or having protein sparing effect in juveniles (Sá *et al*., 2008a). Increasing dietary lipid level from 12 to 18% depressed fry growth (Sá *et al*., 2006). Sharp snout sea bream juveniles (*Diplodus puntazzo*), growth increased as dietary protein level increased up to 45% and was unaffected by dietary lipid level (Coutinho *et al*., 2012). Anyhow, protein sparing effects are still controversial between specialists (Valente *et al*., 2011).

Feed intake and utilization

Feed intake and utilization are tabulated in Table (2). The amount of feed consumed is one of the most important factors for the growth, one of the first objectives was to test whether feed intake was controlled by lipid or protein levels in the diet. Feed intake decreased linearly with increasing lipid level in diets. Fish fed the lowest lipid level diet (10%) consumed 14.97 g/fish, flowed by fish fed diet 15 and 20% lipid level (14.09 and 11.98 g/fish), respectively. Dietary protein levels had no significant effects on the amount of feed consumed.

Dietary lipid levels had significant effects on FCR ($P < 0.001$). The better ratio of FCR was obtained from fish fed 10L diet, the difference was significant. Dietary protein levels had significant effects on FCR ($P < 0.018$). At 50% dietary protein level, fish consumed much less feed against gain in weight recorded. The interactions between dietary protein and lipid levels did not observe any significant difference on FCR.

Digestible energy is thought to be one of the major criteria controlling feed intake in fish (Lupatsch *et al*., 2001) along with other factors including fish size, temperature or palatability. Fish, like homoeothermic animals, seem to adjust feed intake in order to satisfy their digestible energy requirements with their growth rates (Kaushik and Medale, 1994). The best FCR in the present study was achieved for fish fed 15% lipid with 45% protein.

These results are in agreement with those cited for European sea bass (IW < 13 g) by Tibaldi *et al*. (199l) who did not obtain significant differences in growth or FCR rates with diets of 44 and 49% CP compared to higher levels (52 and 56%). Adamidou *et al*. (2011) observed that feed efficiency was negatively affected by dietary lipid level in sharp snout sea bream. However, feed efficiency improved with dietary protein level but was unaffected by dietary lipid level (Coutinho *et al*., 2012). White sea bream feed efficiency was unaffected by dietary lipid levels and improved with dietary protein content (Sá *et al*., 2008 a and b).

Assessment of nutrients retention

Nutrients retention is showed in Table (3). Dietary lipid levels had significant effects on protein efficiency ratio (PER) ($P < 0.001$). The highest protein efficiency ratio (PER) was recorded by fish fed 15L diet (1.42). However, dietary protein levels and the interactions between dietary lipid and protein levels had no significant effects on PER.

This phenomenon has been observed in trout (Cardenete, 1983), red sea bream (Takeuchi *et al*., 1991), European sea bass (Metailler *et al*., 1981), gilthead sea bream (Vergara *et al*., 1999) and red porgy (Schuchardt *et al*., 2008). Wide variations in PER were reported for Malaysian mahseer (Ng *et al*., 2008), juvenile swordtail (Kruger *et al*., 2001), silver dollar (Singh *et al*., 2007) and red porgy (Schuchardt *et al*., 2008). The PER obtained in the present study (1.02-1.47) is higher or within the range. PER significantly dropped as dietary protein increased at the same level of lipid (Coutinho *et al*., 2012), probably due to utilization of more dietary protein as energy when high protein diets are fed to fish. Similarly, PER values dropped as dietary lipid increase at the same level of protein (Sá *et al*., 2006).

Enes *et al*. (2010) found that PER was 1.01 when gilthead sea bream (*Sparus aurata*) juvenile fed isonitrogenous (50% crude protein) and isolipidic (16% crude lipid) diets were formulated to contain 20% of pregelatinized maize starch. Viola *et al.* (1981) reported that both energy and protein retentions are improved if fat is added at the same level of protein, which is used in preference to build body fat, thereby "sparing protein". This well known effect has an energetic aspect; the transformation of protein into fat (in the absence of dietary fat source, carbohydrates or proteins are transformed into body fat, which explains the low retention rate at higher level of protein) was wasteful in terms of energy, because carbon residues of amino acids have to be degraded and fatty acids synthesized from acetyl-Co A. Part of the nitrogen is excreted at further loss of energy. However fat is assimilated into body fat with little degradation and with a minimal loss of energy. In the same trend, Brauge *et al.* (1994) found that protein and energy retention efficiencies tended to increase with decreasing dietary carbohydrate/ lipid ratio. However, they suggested that total body lipid gain in relation to digestible lipid intake was elevated in fish fed diets with the highest digestible carbohydrate levels.

Both dietary lipid and protein levels had significant effects on protein retention (PR). The interactions between dietary lipid and protein levels were not revealed any significant differences on PR. The highest PR was listed for fish fed 15L/45P diet (28.43 %).

Martinez-Liorens *et al*. (2007) found that crude protein efficiency % (PE) and gross energy efficiency % (GE) was 22.3 and 27.9 %, respectively, when *Sparus aurata* fed isonitrogen and isoenergetic diets (46% protein, 14% lipid). In the present study, PR values (20.81–28.43%) were in the range of those reported for other carnivorous marine fish species, such gilthead sea bream (Vergara *et al.*, 1996) and European sea bass (Lanari *et al*., 1993). They were better than those reported for the same species by Espino´s *et al*. (2003).

The PR figure reported here in agreed with those reported by Coutinho *et al*. (2012) who observed that in sharp snout sea bream protein efficiency was inversely related to dietary protein level and improved with dietary lipid level, denoting a protein sparing by dietary lipids. This protein sparing by dietary lipids is confirmed by N retention data (% N intake), which was higher in the high lipid diets (Bureau and Hua, 2010). White sea bream dietary lipids had no protein sparing effect, either in low protein (Ozório *et al*., 2006) or in adequate protein diets (Sá *et al*., 2006, 2008b). The data suggested that nitrogen excretion, resulting from protein catabolism for energy, can be reduced by increasing the non protein energy content of the diet (Couto *et al*., 2008).

Dietary lipid levels had significant effects on energy retention (ER) ($P < 0.001$). The lowest ER was recorded for fish fed 20L diet (19.80%). Dietary protein levels had no significant effects on ER. The interactions between dietary lipid and protein levels had significant effects on ER. Fish received 15L/45P diet was recorded the highest ER (27.27%), while the lowest was recorded for fish fed 20L/45P and 20L/50P being, 19.34 and 20.27, respectively, without significant difference.

Dietary lipid levels had significant effects on phosphorus retention (Phs R) ($P < 0.001$). The highest Phs was produced by fish fed 15L and 20L diets being, 32.44 and 35.02%, respectively, with no significant difference. The lowest Phs was figured for fish fed 10L diet. Dietary protein levels had not effects on Phs. The interactions between dietary lipid and protein levels had significant effects on Phs. Fish fed 15L/45P diet was listed the highest Phs.

Curvilinear regression was used to describe the relationship between FBW and protein intake (PI) (Table 4). Linear regression analysis showed that, FBW increased in a linear pattern with increasing PI ($R^2 = 0.68$). A second-order polynomial model best fitted FBW (g/fish) to PI (g/fish) (Table 4). Based on this model, there was quadratic effect due to varying protein intake on FBW ($R^2 = 0.88$). The polynomial regression analysis showed that, maximum FBW was observed when the PI was 7.75 g/fish.

The relationship between protein retention (PR) and protein intake (PI) was described by linear and a second order polynomial model (Table 4). Linear regression analysis showed that, PR not affected by PI (R^2= 0.09). Quadratic regression analysis showed that, there was a strong correlation between PR and PI ($R^2 = 0.71$) and this implies that the relationship increased when reached the optimum level, and then decreased. According to this model, maximum PR was achieved by fish which consumed 6.50 g PI.

The relationship between ER and PI was illustrated by linear and quadratic regression analysis (Table 4). Linear regression analysis showed that, ER not affected by PI ($R^2 = 0.49$). The polynomial regression analysis showed that, there was quadratic effect on ER (R^2= 0.78) and maximum ER was achieved by fish which consumed 7.35g PI.

The relationship between PhsR and lipid intake (LI) was illustrated by linear and quadratic regression analysis (Table 4).

Table 3. ***Effect of different dietary protein and lipid levels and their interactions on nutrients retention of gilthead sea bream (Sparus aurata L.).***

Treatment	PER[2]	PR[3]	ER[4]	Phs R[5]
Lipid (% DM)				
10L	1.28 b	24.66 b	24.71 b	21.60 b
15L	1.42 a	27.56 a	26.55 a	35.02 a
20L	1.02 c	20.99 c	19.80 c	32.44 a
SEM [1]	0.019	0.417	0.334	0.805
P ANOVA	< 0.001	< 0.001	< 0.001	< 0.001
Protein (% DM)				
50P	1.22	23.56 b	23.91	28.86
45P	1.27	25.25 a	23.46	30.51
SEM	0.016	0.340	0.273	0.657
P ANOVA	> 0.060	< 0.013	> 0.289	> 0.126
Lipid × protein (%DM)				
10L/50P	1.26	23.17	25.63 a	21.30 c
10L/45P	1.31	26.15	23.79 b	21.89 c
15L/50P	1.38	26.70	25.84 a	31.52 b
15L/45P	1.47	28.43	27.27 a	38.51 a
20L/50P	1.02	20.81	20.27 c	33.75 b
20L/45P	1.03	21.18	19.34 c	31.13 b
SEM	0.027	0.589	0.473	1.138
P ANOVA	> 0.325	> 0.168	< 0.033	< 0.015

[1] Pooled standard error of means.
[2]Protein efficiency ratio (PER) = increase in biomass of fish (g)/protein intake (g).
[3]Protein retention (PR) (%) = 100 × protein deposition (g)/protein intake (g).
[4]Energy retention (ER) (%) = 100 × energy deposition (kcal)/energy intake (kcal).
[5]Phs R (Phosphorus retention) = 100× phosphorus deposition (g)/phosphorus intake (g).

Table 4. ***Correlation between growth performance or feed utilization parameters with protein and lipid intake of the experimental diets.***

Regression equations	R^2
FBW $= 2.0014_{PI} - 3.3384$*	0.68
FBW $= -1.4062_{(PI)}^2 + 20.855_{PI} - 65.839$**	0.88
PR $= 1.2857_{PI} + 16.054$*	0.09
PR $= -1.4062_{(PI)}^2 + 20.855_{PI} - 65.839$**	0.71
ER $= 1.2953_{PI} + 1.4881$*	0.49
ER $= -1.3359_{(PI)}^2 + 19.206_{PI} - 57.889$**	0.78
PhsR $= 14.829_{LI} - 0.619$*	0.68
PhsR $= 9.2465_{(LI)}^2 - 20.076_{LI} + 30.957$**	0.69

****Linear regression analysis***
*****Quadratic regression analysis***

Curvilinear regression showed that, there was a strong relationship between PhsR and LI which $R^2 = 0.68$. Based on this model, PhsR was increased with increasing LI. Also, quadratic regression analysis showed that, PhsR was increased with increasing LI ($R^2 = 0.69$). Based on this model, maximum PhsR was achieved by fish which consumed 2.50 g LI.

Chemical body composition

Summarized in Table (5) are the influences of dietary lipid and protein and their interactions on the fish body composition. The interactions between dietary lipid and protein levels did not showed any significant difference on fish body analysis (dry matter, protein, lipid, ash, gross energy and phosphorus contents). Dietary lipid and protein levels had significant effects on dry matter and lipid content. Dietary lipid levels affected significantly on protein content. No significant difference between fish which received 15L and 20L diets on protein content. Dietary protein levels had no significant effects on protein content. Dietary lipid levels had significant effects on ash, gross energy and phosphorus content. However, dietary protein levels had no significant effects on ash, gross energy and phosphorus content.

There was a positive correlation between dietary lipids and carcass lipid contents. Vergara *et al*. (1996) compared the lipid contents of eviscerated and non-eviscerated fish and suggest that fat was increasingly incorporated both in visceral and non-visceral tissues as dietary lipid increased. This may indicate an undesirable increment of fat in fish muscle tissues for these treatments. Similarly, Hernandez *et al*. (2001) reported no significant changes of whole-body protein while lipid and dry matter content were higher in low P/E diets. Grigorakis (2007) and Adamidou *et al*. (2011) reported an increase of body fat with dietary lipid. On the contrary, Coutinho *et al*. (2012) observed that whole-body protein content was higher but lipid content was unaffected in fish fed the high lipid diets.

Table 5. ***Effect of different dietary protein and lipid levels and their interactions on body composition of gilthead sea bream (Sparus aurata) (% Wet basis).***

Treatment	Dry matter %	Protein %	Lipid %	Ash %	GE (KJ)[2]	Phosphorus%
Initial %	27.6	15.2	7.2	5.2	6.417	0.85
Lipid %						
10	32.37 b	17.17 a	10.30 c	4.15 b	8.10 b	0.67 b
15	33.32 ab	16.65 b	11.40 b	5.27 a	8.42 a	0.76 a
20	34.15 a	16.30 b	11.80 a	6.05 a	8.50 a	0.77 a
SEM[1]	0.327	0.115	.082	0.27	0.485	0.011
P ANOVA	< 0.024	< 0.005	< 0.001	< 0.008	<0.002	< 0.001
Protein %						
50	32.71 b	16.78	11.03 b	5.07	8.31	0.73
45	33.85 a	16.63	11.30 a	5.25	8.37	0.74
SEM	0.267	0.094	0.067	0 .226	0.396	0.009
P ANOVA	< 0.024	> 0.300	< 0.030	> 0.588	>0.296	> 0.412
Lipid×protein						
10L//50P	31.50	17.40	10.20	4.40	8.12	0.66
10L//45P	33.25	16.95	10.40	3.90	8.08	0.67
15L//50P	32.85	16.65	11.30	4.90	8.39	0.73
15L//45P	33.80	16.65	11.50	5.65	8.46	0.79
20L//50P	33.80	16.30	11.60	5.90	8.42	0.79
20L//45P	34.50	16.30	12.00	6.20	8.58	0.75
SEM	0.463	0.162	0.115	0 .392	0.69	0.016
P ANOVA	> 0.532	> 0.343	> 0.630	> 0.339	>0.382	> 0.056

[1] Pooled standard error of means.

[2] GE (g/100g): Gross energy was calculated using conversion factors of 39.71 and 23.41 and KJ g^{-1} for fat and protein, respectively,(KJ=4.18Kcal) (Young et al., 2005).

Assessment of water quality under flow-through system

All measured water quality parameters were presented in Table (6). Temperature (°C) was ranged from 23.0 to 24.5 and was not different across treatments. pH was relatively constant all the feeding period. In general, there was no significant difference between treatments on NH_3, NO_2 and NO_3. Fish fed 20L/50P diet produced the highest NH_3 and NO_2 numerically and the lowest NO_3, without significant difference between those fed the other diets. Fish fed 20L/50P diet had high significant on PO_4 compared to other diets.

Effluents from flow-through systems can be characterized as continuous, high-volume flows containing pollutant concentrations. In the present study, the effluents from flow-through systems were affected by the dietary lipid and protein levels. The higher nutrients levels effluent recorded for control 20L/50P diet indicate that the protein sparing effect of lipids may be an effective strategy to decrease the environmental impact for the sea bream effluent under flow-through system (Sindilariu, 2007). Rychly (1980) observed a decrease of 45% N excretory losses when dietary protein decreased from 74% to 32% with corresponding increase of dietary carbohydrate from 9% to 53%. Kaushik and Cowey (1991) found that dietary energy is supplied from non-protein contents, such as lipid and carbohydrate, resulting in the reduction of amino acid (AA) catabolism for energy and reduction in nitrogenous wastes and showed that N excretion was decreased when digestible energy increased in the form of either fats or digestible carbohydrates.

First solution to reduce the environmental impacts of aquaculture systems consists in minimizing the FCR: a 30% reduction of FCR in a trout farm resulted in a reduction of almost 20% of the global environmental impact, excluding energy use (Roque d'Orbcastel *et al*., 2009). The development in salmonid diet is a good example of these improvements, which saw a 50% increase in growth rates, lipid content in salmonid diets has increased from 15-20% to 30-35% and the phosphorus content has decreased from 2.0 to 1.0% (Bureau and Hua, 2010; Sarker *et al*., 2013). Protein sparing by dietary lipid has been shown to occur in most fish species, which is an effective method for the management of nitrogenous waste in fish culture (Cho and Bureau, 2001).

CONCLUSION

Considering the obtain data, it could be conclude that, 15L/45P diet satisfies the growth requirements of *Sparus aurata* fingerlings to ensure minimum environmental impact and may be high economic efficiency under flow-through system.

Table 6. ***Effect of different dietary protein and lipid levels on water quality for gilthead sea bream (Sparus aurata L.) under flow-through system (mg/L).***

Treatment	pH	Temp	NH_3	NO_2	NO_3	PO_4
10L/50P	7.62±0.03	23.5±0.2	0.24±0.25	0.06±0.05	1.20±0.02	0.04±0.02^{b}
10L/45P	7.55± 0.03	23.4±0.2	0.11±0.00	0.04±0.00	1.05±0.22	0.04± 0.01^{b}
15L/50P	7.60± 0.01	24.5±0.1	0.11±0.01	0.05±0.01	1.00±0.03	0.03± 0.01^{b}
15L/45P	7.64± 0.04	23.3±0.5	0.12±0.01	0.02±0.01	1.03±0.03	0.04± 0.01^{b}
20L/50P	7.60± 0.01	23.0±0.5	0.38±0.01	0.06±0.02	1.02±0.12	0.08± 0.01^{a}
20L/45P	7.58± 0.01	23.0±1.0	0.12±0.01	0.00±0.02	1.33±0.00	0.02± 0.01^{b}
P (ANOVA)	> 0.44	> 0.424	> 0.42	> 0.44	> 0.29	< 0.04

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أستفادة اسماك الدنيس من البروتين والدهن مع اعتبار الأثر البيئي تحت نظام المياه المتدفقة

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أجريت تجربة عملية لمدة ٥٨ يوم لتقييم مستويات مختلفة من البروتين والدهن علي أداء أسماك الدنيس مع الأخذ في الإعتبار الأثر البيئي تحت نظام المياه المتدفقة . تم تصميم تجربة عملية مكونة من ثلاثة مستويات من الدهن وهي ١٠ ، ١٥ ، ٢٠ % علي أساس المادة الجافة ومستويين من البروتين وهما ٥٠ ، ٤٥ % علي أساس المادة الجافة في تصميم عاملي(٣×٢). تم خلط ستة علائق مختلفة في محتواها من مستويات البروتين والدهن وغذيت بها أسماك الدنيس- متوسط وزنها ١,٥ جرام - ثلاثة مرات يوميا حتي الشبع ولمدة ٥٨ يوم وكانت النتائج كما يلي :

مستويات الدهن بالعليقة أثرت معنوياً علي الوزن النهائي للأسماك ، الغذاء المأكول ، معامل التحويل الغذائي ، كفاءة البروتين ، البروتين المحتجز بجسم الأسماك ، الفوسفورالمحتجز والطاقة المحتجزة بجسم الأسماك . مستويات بروتين العليقة أثرت معنوياً علي الوزن النهائي للأسماك ، معامل التحويل الغذائي ، البروتين المحتجز بجسم السمكة ولكن لم تؤثر معنوياً علي الغذاء المأكول، كفاءة البروتين ، الطاقة المحتجزة والفوسفور المحتجز بجسم السمكة . التداخل بين مستويات البروتين والدهن أثرت معنوياً علي الوزن النهائي ، الطاقة المحتجزة والفوسفور المحتجز بجسم السمكة ولكن لم يؤثر هذا التداخل معنوياً علي الغذاء المأكول ، معامل التحويل الغذائي ، كفاءة البروتين والبروتين المحتجز بجسم السمكة. الأسماك التي غذيت علي العليقة التي بها ١٥٠ جرام دهن و٤٥٠جرام بروتين لكل كيلو جرام أظهرت معنوية أفضل من المعاملات الاخري علي الوزن النهائي للأسماك والطاقة والفوسفور المحتجز بجسم الأسماك . نتائج هذه الدراسة تشير إلي أن العليقة التي بها ١٥٠ جرام دهن و٤٥٠جرام بروتين لكل كيلو جرام كافية للحصول علي أفضل معدل نمو لأسماك الدنيس بما يضمن أقل أثر بيئي ممكن وربما تحقق كفاءة أقتصادية عالية تحت نظام المياه المتدفقة.