

Growth, Feed Utilization and Body Composition of White Sea Bream *Diplodus sargus* Juveniles Offered Diets with Various Protein and Energy Levels

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Abstract. White sea bream *Diplodus sargus* is a valued table fish in the Mediterranean region and elsewhere (Black Sea and Eastern Atlantic), and is a candidate aquaculture species in various countries. Understanding dietary protein requirements and protein to energy ratios is imperative for development of a successful culture of this species. A ten week experiment was performed to estimate growth performance of *D. sargus* juveniles offered diets with various levels of protein and energy. Six diets were formulated to contain three levels of protein (25, 35 and 45%) plus two levels of lipids (8 and 12%), and were offered to white sea bream juveniles in triplicate tanks, three times daily, to satiation. Survival was greater than 93% in all treatments. Growth increased with increasing dietary protein and increasing lipid content of the diets. Feed intake increased with increasing dietary protein but was not affected by dietary lipid levels. Protein efficiency decreased with increasing dietary protein levels. Moisture, protein and ash levels in the fish were not affected by dietary protein but body lipid content increased with increasing dietary protein. Dietary protein levels seemed to affect red blood cells. A greater proportion of immature red blood cells were found in blood of fish offered the 25% and the 45% protein diets than in blood of fish offered the 35% protein diet. Results of the present work suggest that a 45% protein, 12% lipid diet results in good growth of *D. sargus*

juveniles but might affect blood composition and thus be detrimental if offered to the fish for a long period of time.

Keywords: white sea bream, *Diplodus sargus*, protein requirement, dietary energy level

Introduction

Aquaculture of fresh water fish, mainly tilapia, is quite successful in Egypt. Marine aquaculture is less well developed and consists almost exclusively of sea bass, *Dicentrarchus labrax* and sea bream, *Sparus auratus*. Developing aquaculture protocols for other high value fish species in order to diversify and improve fish farming is thus very important (El-Dakar, 1999; and Dores *et al.*, 2000). White sea bream, *Diplodus sargus* has good market demand in Egypt and internationally, with prices on par with those of *S. auratus* (Papageorgiou, 2000). White sea bream is a valued table fish in the Mediterranean region and elsewhere, and is a candidate aquaculture species in various countries (Cejas *et al.*, 1993; and Abellan and Garcia-Alcazar, 1995).. It is an omnivore and feeds low on the food chain, (Figueriedo *et al.*, 2005), it adapts easily to captivity (Sa *et al.*, 2006), and its juvenile growth rate is similar or even exceeds that of gilthead sea bream (Cejas *et al.*, 1993; and Abellan and Garcia-Alcazar, 1995). Additionally, white sea beam culture protocols resemble those of other sea bream species, especially at larval and fingerling stages (Cejas *et al.*, 1993; and Abellán and Basurco, 1999).

Studies that determine nutritional requirements of candidate aquaculture species are essential for development of culture protocols (Robaina and Izquierdo, 2000). Knowledge of dietary protein and energy requirements for fish is thus a primary consideration when formulating fish diets (Lee and Kim, 2001). Increasing protein level in diets can lead to improved fish production, especially for carnivorous fish although, excessive dietary protein is not economical for fish culture. Improper protein and energy levels and/or improper protein to energy ratios will result in an increase in fish production cost and a deterioration in water quality (Yang *et al.*, 2002). Insufficient energy in diets reduces protein efficiency due to increased dietary protein use for energy and concomitantly increases ammonia production thus affecting water quality (El-Dakar, 1994; Shyong *et al.*, 1998; Shalaby, 1998; and El-Dakar *et al.*, 2003). Conversely, excessive energy in diets can lead to increased body

lipid deposition and growth reduction due to possible reduction of necessary nutrients for growth (El-Dakar, 1994; and Van der Meer *et al.*, 1997; Shyong *et al.*, 1998; Shalaby, 1998; and El-Dakar *et al.*, 2003). Accordingly, the optimization of protein to energy ratio in diets plays an important role in protein and energy utilization in fish (Kaushik, 1994; and Lee and Kim, 2001).

Energy in marine fish diets is generally increased by increasing lipid content of the feed. However, Sa *et al.* (2006) found that excessive lipid in *D. sargus* diets caused a reduction in growth while Ozorio *et al.* (2006) found no effect of lipid level on growth performance. Studies varying both dietary protein and energy levels have demonstrated fish capability to spare protein when non-protein energy sources (NPES), lipid and carbohydrate, are added to the diet (McGoogan and Gatlin, 1999, 2000; Nankervis *et al.*, 2000; Shalaby *et al.*, 2001; Meyer and Fracalossi, 2004; and Kim and Lee, 2005). However, the use of NPES in fish diets must be properly evaluated because excessive use results in reduction of feed intake, deposition of lipids, and inhibits the utilization of other nutrients (Ai *et al.*, 2004). Accordingly, use of proper P/E ratio should be taken into account when fish diets are formulated. White sea bream appear to be able to grow very well using a wide range of dietary protein, starting from as low as 25% (Sa *et al.*, 2002, 2006, 2007, 2008). Nevertheless, information on protein to digestible energy ratios necessary for optimal growth of *D. sargus* juveniles is lacking.

A few studies have been performed to study nutritional requirements of white sea bream (Sa *et al.*, 2006, 2007; and Ozorio *et al.*, 2006). Based on findings by these authors, the present work was designed to study the effect of a narrow range of dietary protein inclusion at two energy levels, on growth, feed utilization, and body composition, of white sea bream juveniles.

Materials and methods

The present work was performed at the Fish Nutrition Lab., Aquaculture Division, National Institute of Oceanography and Fisheries (NIOF) in Alexandria, Egypt. Six experimental diets were formulated to contain three levels of protein (25, 35 and 45%) plus two levels of lipids (8 and 12%). Calculated digestible energy of the diets ranged from 16.1 to 17.6 kJ/g. Ingredient composition of the experimental diets is

presented in Table (1). Fishmeal was produced locally from small “trash” fish purchased at the fish market. Fish were oven dried at 60 °C for 48 hours then ground, sieved and stored at -20 °C until use as an animal protein source. Soybean meal (44%) was used as a plant protein source. Yellow corn, corn starch and sunflower oil served as energy sources. The experimental diets were prepared by mixing dry ingredients with boiling water. The mash was then extruded in a meat grinder through a 1 mm die, and pellets air dried to less than 10% moisture and stored at -20 °C.

Eighteen 70-L glass aquaria (70 × 40 × 30 cm) were used in the study. All aquaria were filled with filtered sea water. Approximately 35% of the water was exchanged daily and aeration was provided using submerged air diffusers and a reciprocating air blower. Photoperiod was maintained at 12:12 L:D. Salinity, temperature, pH, and dissolved oxygen were measured daily and remained at approximately 35‰, 27 ± 2°C, and 7.8 ± 0.2, 6.8 ± 0.3 mg/kg, respectively. Ammonia and nitrite nitrogen were measured periodically using a LaMotte saltwater aquaculture kit and remained below 0.2 mg/L.

Table 1. Ingredients and proximate analysis of experimental diets.

Ingredients %	Diet No.					
	1	2	3	4	5	6
Fish meal	19	19	34	34	48	48
Soybean meal	25	25	25	25	25	25
Yellow corn	15	15	15	15	15	15
Corn starch	34.85	30.85	21.25	17.25	8.56	4.56
Fish oil	4.15	8.15	2.75	6.75	1.44	5.44
Vitamin & mineral mix ¹	2	2	2	2	2	2
Dry matter	97.99	98.89	98.89	99	98.78	98.86
% DM basis						
Crude protein	25.25	25.3	35.75	35.8	45.55	45.6
Total lipid	7.98	11.99	8	12.01	8.02	12.02
Crude Fiber	3.55	3.51	3.55	3.54	3.57	3.55
Ash	6.77	6.67	8.57	8.55	10.31	10.26
Carbohydrates	56.45	52.53	44.13	40.1	32.55	28.57
GE (KJ/g) ²	19.1	20.0	19.4	20.3	19.7	20.6
DE (KJ/g) ³	16.7	17.6	16.4	17.3	16.1	17.0
P/DE (g/KJ)	1.5	1.4	2.2	2.1	2.8	2.7

¹ Eco Vit, Egyptian Veterinary products and feed additives Co., Demyatta, Egypt. The vitamin and premix provided the following per kg of experimental diet: 15 000 IU, 0.7 g, 15 000 IU, 2 mg, 2.5 mg, 2 mg, 10 mg, 3 mg, 5 mg, 2 mg, 2 mg, 5.5 mg, 200 g, 90 g, 40 g, 2.5 g, 48 g, 3.6 g, 23.5 g, 8 g, 450 mg, 200 mg and 20 mg of vitamin A, vitamin C (Stay C₂, 35% active), vitamin D3, vitamin E, vitamin B2, vitamin K3, nicotinic acid, vitamin B6, vitamin B12, vitamin B1, folic acid, Ca-D-pantothenate, calcium, phosphate, sodium, copper, magnesium, manganese, zinc, iron, cobalt, iodine and selenium.

² GE = Gross energy in the diet.

³ DE = Digestible energy.

Juveniles of *D. sargus* were caught in a seine net off the coast of Alexandria, Egypt and transported live to a large fiberglass tank filled with seawater. Fish were maintained in the tank for two weeks and offered a commercial fish feed twice daily to apparent satiation. At the start of the experiment, fish were sorted manually to similar size. Ten fish (average body weight, 0.57 g/fish) were randomly distributed into each of the aquaria. During the first three days of the experiment, dead fish were replaced with individuals of the same size. A 3×2 factorial design with three randomly chosen replicate tanks per dietary treatment was used. The feeding trial lasted 10 weeks and the fish were fed by hand three times daily (9.00, 12.00 and 15.00 hrs) to apparent visual satiation, 6 days a week. Fish were weighed biweekly after one day of fasting.

Diets and fish samples were analyzed according to AOAC (1990). At the end of the experiment, five fish from each tank were randomly removed and dried at 60 °C for 48 hrs to estimate moisture content. Dried samples were then finely ground and stored at -20 °C. Samples were analyzed for protein content using the micro-Kjeldahl method ($N \times 6.25$) and lipids extracted using the Soxhlet method with ethyl ether as solvent. Ash content was determined by burning the samples in a muffle furnace at 600 °C for two hrs. Gross energy content of experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.22 kcal/g for protein, lipid and carbohydrate, respectively (NRC, 1993). Digestible energy content was estimated from standard physiological fuel values as 4, 9 and 4 kcal/g for protein, carbohydrate and lipid, respectively (Garling and Wilson, 1976).

Blood samples were collected directly from caudal artery after severing the caudal peduncle. Thin smears were dried, fixed with methyl alcohol and stained with Giemsa stain. Erythrocytes were examined for variation in size and shape using a light microscope (X100). Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency (PE), protein retention efficiency (PRE) and energy retention (ER) were calculated as:

$$\text{SGR} = 100 \times (\ln \text{FBW} - \ln \text{IBW}) / t$$

where FBW is final body weight (g), IBW is initial body weight (g) and t is time in days.

$$\text{FCR} = \text{weight of feed offered to fish (g)} / \text{weight gain of fish (g)}$$

$$\text{PE} = \text{weight gain of fish (g)} / \text{protein fed (g)}$$

$$\text{PRE} = [\text{protein retained (g)} / \text{protein fed (g)}] \times 100$$

$$\text{ER} = [\text{energy retained in fish (J)} / \text{energy offered to fish (J)}] \times 100$$

All statistical analyses were performed using MSTAT-C. Analysis of variance (ANOVA) was carried out according to Snedecor and Cochran (1982) using two-way ANOVA with fixed-effect model and parameters with significant differences among treatment means were subjected to Duncan's Multiple Range-Test ($\alpha = 0.05$) (Duncan, 1995).

Results and Discussion

Survival and growth parameters of white sea bream offered the experimental diets for 10 weeks are shown in Table (2). Survival was greater than 93% in all tanks and there were no significant differences ($P > 0.05$) among treatments. Specific growth rates of the fish ranged between 3.7 and 4.2 %/d, similar to results reported by Sa *et al.* (2006) for *D. sargus* with an initial weight of 1.5 g but much greater than SGR reported by Sa *et al.* (2006, 2007, 2008) when the initial weight of the fish was more than 14g. Our results corroborate statements by these authors that white sea bream grow rapidly when very small but growth slows significantly when fish are larger.

Table 2. Effect of different dietary protein and oil levels on growth and survival of white sea bream *Diplodus sargus* juveniles.

Diet no.	CP	Lipid	Initial Wt. (g/fish)	Final Wt. (g/fish)	Wt. Gain (g/fish)	SGR (%/day)	Survival %
1	25	8	0.57	7.90 ^f	7.34 ^f	3.77 ^f	96
2		12	0.56	8.35 ^e	7.79 ^e	3.86 ^e	93
3	35	8	0.56	9.10 ^d	8.54 ^d	3.99 ^d	100
4		12	0.56	9.55 ^c	8.99 ^c	4.06 ^c	97
5	45	8	0.56	10.05 ^b	8.19 ^b	4.13 ^b	100
6		12	0.57	10.95 ^a	10.39 ^a	4.24 ^a	96
Protein level	P			0.001	0.001	0.001	0.17
Energy level	E			0.001	0.001	0.001	0.34
Interaction	PxE			0.051	0.054	0.001	0.37
PSE*				0.08	0.08	0.03	2.60

* PSE = Pooled Standard Error

Final body weight, and SGR of fish in the present experiment were significantly influenced by both dietary protein and energy levels ($P < 0.01$); both measures improved as dietary protein and energy levels increased. Growth of white sea bream increased with increasing dietary protein from 25 to 45%, suggesting that crude protein requirements for maximum growth for white sea bream may be at or more than 45% of the diet. These results are not supported by previous studies by Sa *et al.* (2006, 2008). Sa *et al.* (2006) found that a 38% protein diet was sufficient for proper growth of *D. sargus* and Sa *et al.* (2008) reported that 27% dietary protein was sufficient for maximal growth.

Dietary lipid also affected weight gain. However, because Sa *et al.* (2006) and Ozorio *et al.* (2006) reported that dietary lipid had no effect on white sea bream growth, we have to assume that growth difference observed among treatments of the present experiment must be due to the small differences in dietary energy densities between the 8% lipid diets and the 12% lipid diets. This suggests that increasing dietary energy possibly provides for a more efficient utilization of dietary protein by white sea bream. Similar improvements of fish protein utilization with increasing dietary energy were observed in rabbitfish *Siganus rivulatus* by Shalaby *et al.* (2001) and in red snapper *Lutjanus campechanus* by Miller *et al.* (2005). Sa *et al.* (2007) showed that dietary energy for *D. sargus* could be obtained from carbohydrates rather than lipids, and that fish offered iso-energetic diets grew similarly when diets contained 64% protein and no carbohydrates, or 38% protein and 36% carbohydrates.

Feed intake, FCR and protein efficiency of white sea bream offered the experimental diets are shown in Table (3). Feed intake was affected by dietary protein level ($P > 0.05$) but not by lipid level. There was no interaction between protein level and energy density of the diet with regards to feed intake. However, the data seem to suggest that protein affected feed intake only at low lipid levels. Feed intake decreased at low protein and lipid levels which contradicts findings by Ozorio *et al.* (2006) that *D. sargus* ingest more food when dietary protein levels decrease. Sa *et al.* (2008) state that fish consume less feed when diets are not balanced but find that white sea bream increase feed intake to compensate for low protein density of feeds. This suggests that our low protein/low lipid diet was not a balanced nutritional source for the fish. The reason is probably the low protein to carbohydrate level of the diet (see Sa *et al.*, 2007).

Table 3. Effects of various dietary protein and energy levels on feed and nutrient utilization of white sea bream *Diplodus sargus* juveniles. (CP = Crude protein; FI = Feed intake in g/fish; FCR = Feed conversion ratio; PE = Protein efficiency; PR = % Protein retention; ER = % Energy retention)

Diet	CP	Lipid	FI	FCR	PE	PR	ER
1	25	8	7.36 ^b	1.66 ^a	2.40 ^a	44.99 ^a	20.78 ^{c,d}
2		12	8.51 ^a	1.76 ^a	2.25 ^a	37.99 ^b	18.79 ^d
3	35	8	8.44 ^a	1.51 ^b	1.86 ^b	32.40 ^{b,c}	23.13 ^{b,c}
4		12	8.79 ^a	1.46 ^b	1.93 ^b	34.95 ^b	24.75 ^{b,c}
5	45	8	9.45 ^a	1.44 ^b	1.53 ^c	26.70 ^c	25.14 ^{a,b}
6		12	9.25 ^a	1.24 ^c	1.78 ^b	32.68 ^{b,c}	28.88 ^a
Protein level P			0.006	0.006	0.001	0.005	0.002
Energy level E			0.105	0.29	0.32	0.26	0.27
Interaction PxE			0.128	0.49	0.07	0.12	0.10
Pooled standard error			0.28	0.05	0.06	1.80	1.12

Protein efficiency decreased with increasing dietary protein level, but was not influenced by dietary energy levels ($P > 0.05$). A decrease in protein retention efficiency with increasing dietary protein was expected and is frequently reported in the literature (see Cho *et al.*, 2001; and Ozorio *et al.*, 2006). Statistically, PRE was not affected by energy density. However, when treatments with 35% protein were removed from the data set, a significant effect of dietary energy on protein retention was detected. Data in Table 3 suggest such an effect even though statistically not shown. Reports of dietary energy effects on protein retention in various other sea breams are mixed, with some authors reporting no effect while others reporting an obvious effect (Company *et al.*, 1999; Santinha *et al.*, 1999; Vergara *et al.*, 1999; Espinos *et al.*, 2003; Skalli *et al.*, 2004; and Ozorio *et al.*, 2006). Results of the present experiment suggest that feed energy density has a sparing effect on proteins but the energy can be of a carbohydrate source rather than a lipid source. Energy retention was not significantly ($P > 0.05$) affected by dietary lipid levels but did increase with an increase in dietary protein. These results were expected because protein content of the various feeds varied much more than lipid content and the total caloric value from protein in the feeds was greater than the caloric content of lipids.

Moisture, protein and ash contents of fish carcasses were not affected by neither dietary protein nor dietary lipid levels. However, fat content of fish tended to increase with increasing dietary protein level. Similarly, Sa *et al.* (2006) reported no effect of dietary protein on whole body protein proportion of white sea bream while Sa *et al.* (2008) observed a slight effect when diets contained 48.7% protein. Ozorio *et al.*

(2006) reported an effect of dietary lipids on body protein content. The discrepancy among results of various experiments is most probably because of differences in fish size, nutritional history, dietary composition *etc.* Nevertheless, to most aquaculturists, the proximate composition of fish can vary within reason without affecting marketability of the product. Protein efficiency and protein retention are more important variables affecting economic returns.

Lipid content of the fish was affected by protein levels in the diet (Table 4). Similar results were observed by Ozorio *et al.* (2006), but not by Sa *et al.* (2006, 2007, 2008). However, data from Sa *et al.* (2008) suggest a decrease in body lipid proportion as protein to energy ratio (P/E) of the diet increased. Results of the present work suggest an increase in body lipids with an increase in dietary P/E. Again we believe the discrepancies among results are because of various factors listed above and more work needs to be done before conclusions are made.

Table 4. Effects of various dietary protein and energy levels on body composition of white sea bream *Diplodus sargus* juveniles.

Diet	Dietary protein	Lipid	Moisture	Protein	Lipid	Ash
Initial fish			75.00	15.51	4.25	5.16
1	25	8	69.57	17.02	6.20 ^b	5.72
2		12	71.72	16.8	6.35 ^b	5.13
3	35	8	70.6	17.36	6.47 ^b	5.32
4		12	69.25	17.97	7.44 ^a	5.27
5	45	8	69.95	17.37	7.31 ^a	5.02
6		12	68.8	18.28	7.49 ^a	5.29
Protein level	P		0.37	0.25	0.006	0.21
Energy level	E		0.06	0.34	0.05	0.31
Interaction	PxE		0.14	0.21	0.18	0.05
PSE*			0.83	0.535	0.22	0.14

* PSE = Pooled Standard Error

Blood smears of fish offered the 25% protein, 8% fat diet contained an abundance of immature red blood cells. The immature erythrocytes had lightly staining cytoplasm, large nuclei, and thick chromatin threads (Fig. 1). This condition was even more pronounced in fish offered the diet with 45% protein and 8% fat (Fig. 2). However, in fish offered the 35% protein, 8% fat diet, erythrocytes structure appeared normal. Most of the cells were mature, with a homogenous cytoplasm and condensed nuclear material (Fig. 2). In fish offered diets with 12% lipid inclusion, red blood cells became deformed. Blood of fish offered the 25% protein, 12% lipid diet exhibited poikilocytosis, abnormally shaped erythrocytes

(bud-shaped and tear-shaped). At higher dietary protein levels (35% & 45%) fish became anemic, erythrocytes aggregated and lysed, and white blood cell developmental stages increased (Fig. 5 and 6). Vacuoles in blood cells of fish fed protein level of 45% suggested decreased haemoglobin content (Fig. 6).

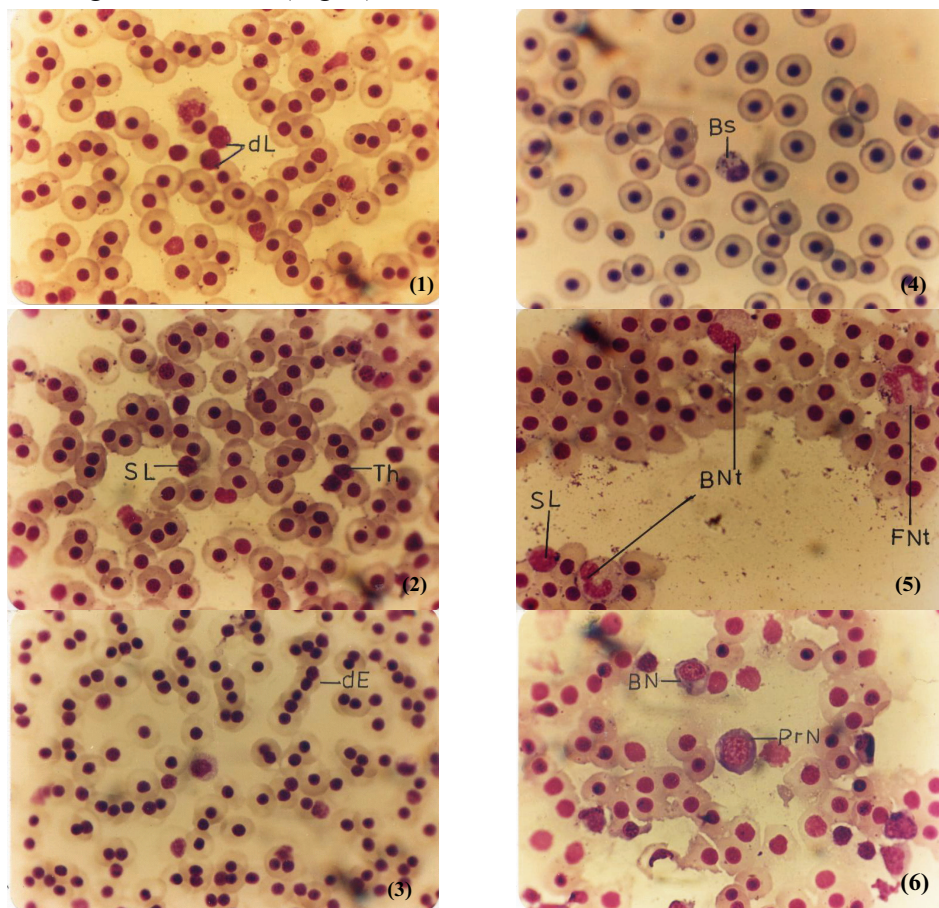


Fig. 1-6. Blood film of fish fed diets of 8% fat $\times 100$. 1: Fish fed diet 25% protein showing, immature erythrocytes; 2: Fish fed diet of 45% protein showing, immature erythrocytes; 3: Fish fed diet of 35% protein showing, mature erythrocytes. (4-6): Blood film of fish fed diets of 12% fat $\times 100$; 4: Fish fed diet of 25% protein showing bud and tear-shaped erythrocytes; 5: Fish fed diet of 35% protein showing aggregated and lysed erythrocytes and increases in granulocytes; 6: Fish fed diet of 45% protein showing, vacuolated erythrocytes. Developing lymphocytes (dL), small lymphocyte (sL), thrombocyte (Th), dividing erythrocyte (dE), Basophilic granulocytes (BS), developmental stage of neutrophilic granulocyte (BNt, FNt) and developmental stage of erythrocyte (PrN).

In conclusion, results suggest that although white sea bream grow best when offered a 45% protein 12% lipid diet, haematological evaluations warn that a high protein and lipid diet might affect blood cells and thus be detrimental to the fish on the long term. Until we understand white sea bream nutritional requirements better, we would suggest offering the fish diets with 35% protein and 8% lipids.

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النمو، الكفاءة الغذائية وتركيب الجسم لأسماك الشراغيش المغذاة على علائق مختلفة في مستويات البروتين والطاقة

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المستخلص. يعتبر سمك السرغوس طبقا شائعا في منطقة البحر الأبيض المتوسط والعديد من الدول الاستوائية، وهذا النوع من السمك مرشحا للتربية المائية في مختلف البلدان. إن فهم متطلبات البروتين والطاقة في غذاء هذه السمكة أمر حتمي لتطوير التربية المائية وجعلها صناعة ناجحة. ولتحديد معايير النمو لأسماك السرغوس الصغيرة، تم إجراء تجربة مدتها عشرة أسابيع منحت خلالها الأسماك وجبات غذائية تتضمّن مستويات مختلفة من البروتين والطاقة، أعطيت خلالها الأسماك ست وجبات غذائية تحتوي على ثلاثة مستويات من البروتين (٣٥، ٢٥، و ٤٥%) ومستويين من الدهون (٨ و ١٢%)، منحت بمعدل ثلاث مرات يوميا حتى الأشباع. كانت نسبة بقاء الأسماك على قيد الحياة أكثر من ٩٣% في جميع الأحواض. ازداد معدل النمو مع ازدياد البروتين الغذائي وازدياد محتوى الدهون الموجودة في الوجبات الغذائية. وقد ازدادت كمية الأكل المستهلكة مع ازدياد البروتين الغذائي لكنه لم يتأثر بمستوى الدهون الموجودة في الغذاء. انخفضت فعالية البروتين مع ازدياد مستوى البروتين الغذائي.

ولم تتأثر كمية المياه، البروتين و مستوى الرماد في لحم الأسماك ولكن محتوى الدهون في الجسم ازداد مع زيادة البروتين. وُجد أن مستوى البروتين في الغذاء له تأثير على كريات الدم الحمراء، فنسبة عدد خلايا الدم الحمراء القليلة النمو ارتفعت في دم الأسماك التي منحت ٢٥ و ٤٥% من البروتين الغذائي أكثر من الأسماك التي منحت ٣٥% من البروتين الغذائي. أظهرت نتائج هذا العمل أنّ ٤٥% من البروتين و ١٢% من الدهون هي النتيجة الأمثل لنمو صغار السرغوس ولكن من جهة ثانية قد تؤثر على تركيبة الدم وبالتالي قد تكون ضارة إن منحت للأسماك لمدة طويلة من الزمن.