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REARING SHARP-SNOUT SEABREAM (*DIPLODUS PUNTAZZO*) FINGERLINGS AT VARYING DIETARY PROTEIN AND LIPID LEVELS

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ABSTRACT

This experiment was carried out to assess the protein sparing effect of dietary lipid on growth performance, feed utilization, carcass composition and estimated cost of production of sharp-snout seabream (*Diplodus puntazzo*). Four supplemented diets formulated to contain 50% protein with 3% lipid, 45% protein with 6% lipid, 40% protein with 8% lipid and 35% protein with 10% lipid. Ten weeks feeding trial was carried out on sharp-snout seabream fingerlings, with a mean weight of 4.38± 0.1 g in eight-glass aquaria. Results showed no significant differences in growth of fish fed diet1 (50% protein and 3 % lipid), diet 2 (45 % P and 6 % L) or diet 3 (40% P and 8 % L), whereas fish fed diet 4, (35% P and 10 % L) showed the least growth rates among all. The best feed conversion ratio (FCR), efficiency of protein utilization in terms of protein productive value (PPV) and protein efficiency ratio (PER) as well as the energy utilization (EU) were achieved with diet 3 which contained 40% P and 8 % L. Carcass lipid level increased from 20.76% for fish fed diet 1 to 24.2% for fish fed diet 4, when dietary lipid level increased from 3 % to 10 % and therefore, the energy retention increased in fish. Also, there was a decrease in the level of carcass crude protein as dietary protein level decreased from 50% in diet 1 to 35% in diet 4. The least cost of kg fish production (9.04 LE/Kg fish), was recorded for fish fed diet 3 (40% protein and 8% lipid) followed by fish fed diet 4 (35%P & 10%L), (9.11 LE/Kg fish). The control diet that contained 50%P &3% L gave the highest cost of kg fish production (10.82 LE/Kg fish), followed by diet 2 (10.63 LE/Kg fish) that contained 45% P & 6% L. It is concluded that a supplemented feed with 40%P and 8%L was the best for feeding Sharp-snout seabream (*Diplodus puntazzo*) fingerlings, and addition of 8% lipid to the diet is sufficient enough as energy source.

INTRODUCTION

Aquaculture is one of the fastest growing food production activities in the world and plays an important role in the world fish production (FAO, 2003). The supply of feed materials is a major expense in aquaculture operations. Protein is the most essential and expensive component in fish feeds. Information on the protein requirements of marine fish is necessary to minimize feed cost inputs. Protein fraction should be optimally utilized for growth rather than for serving as energy source for fish. Information on the protein sparing effect of non-protein nutrients such as lipid are necessary and should be used to reduce feed-cost and maximize nitrogen retention. The importance of lipid as a main source of energy was studied by many authors (Halvar, 1976; Winfree and Stickney, 1981; Viola *et al*., 1988; Kheir, 1997 and El-Ebiary & Mourad, 1998). Tacon, 1997, reported that it must reduce current dependence of farming systems for carnivorous fin-fish species upon fishmeal and fish oil and other food grade fishery resources as feed inputs and use of alternative more sustainable sources of dietary protein and lipid. Protein sources

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should be targeted for evaluation and use should include plant-based protein meals such as oilseeds, pulses and protein concentrate. Although fish oil is an excellent source of n-3 fatty acids for fish, some plant oils, such as linseed oil and soybean oil contain significant quantities of n-3 fatty acids (Lovell, 1994). Moreover, although linseed oil is currently only produced in small quantities, it is a rich source of both 18:3 n-3 and 18:2 n-6 (Tocher, *et al*., 1990). Tacon, 1995; Rumsey, 1994,show that replacing the major part of the marine fish lipid component of commercial aqua feeds with plant lipids as a source of dietary energy for marine finfish rather than using fish oil as an energy source.

Sharp-snout sea bream (*Diplodus puntazzo*) is carnivorous in nature therefore, it would be expected to have a high protein requirement like other carnivorous fish species (Weathereley and Gill, 1987). One of the challenges that face fish nutritionists is to spare the expensive dietary protein with inexpensive non-protein energy source such as lipid. Lipid must be included in fish diets at levels that maximize the use of protein for growth (El-Sayed and Garling, 1988). Since the basic nutrient requirements for Sharp-snout sea bream (*Diplodus puntazzo)* have not been studied as well as the available information concerning the suitability of commercial diets used for rearing other sparid fish such as *Diplodus puntazzo* is scarce. Therefore, The main objective of this work is to determine the suitable level of dietary lipid (linseed oil) that spares the dietary protein for maximum growth, feed utilization, good quality of body composition and cost of production for Sharp-snout Sea bream (*Diplodus puntazzo*).

MATERIALS AND METHODS

This work is carried out through the project of: distribution, biological and rearing young fish of marine water at the area from Alexandria to Damietta, financed by National Institute of Oceanography and Fisheries, Alexandria. The experiment was conducted in Breeding Laboratory, (NIOF), Alexandria, Egypt. Eight glass aquaria, each aquarium (100 X 40 X 30 cm.) having a 120- liter natural seawater (35g / l). The water in each aquarium was provided with sufficient aeration by means of an electric air pump.

Sharp-snout Seabream (*Diplodus puntazzo*) fingerlings were collected alive from the seacoast in front of NIOF, Anfoushy Alexandria by basket trap (local name, Gobia) during May 2005. Fish fingerlings had average total length of 6.5± 0.1cm and average weight of 4.38± 0.1 g. Fish were acclimatized to laboratory conditions for one week prior to the initiation of the experiment, in well natural sea water, and fed at natural food (collected by phytoplankton net). Afterwards, fish were transferred to the experimental aquaria and stocked in duplicates at a density of 10 fingerlings / aquarium. Aquaria were represented four groups. Fish were fed experimental diets formulated to contain about 50% protein with 3% lipid; 45% protein with 6% lipid; 40% protein with 8% lipid and 35% protein with 10% lipid. Feeds were made of locally available ingredients at reasonable price (Table 1). The ingredients were thoroughly mixed and formulated as finely powdered diet. Lipid used was added to warm water and then spraying onto the mixed ingredient. After that the formulated diets put in dry oven at 70^{o} C for 48hr. Diets were packed in airtight plastic bags and stored in a deep freezer at - 20^{o} C until needed. Chemical composition and energy values of the four experimental diets were determined according to AOAC (1990) methods and are shown in Table (1). Fish in each group of aquaria (2) were fed one of the four diets. The diets were offered to fish in a dry form, six days a week at a rate of 5% of the total fish weight, two times a day for 71 days. The amount of feed given to each aquarium was determined at the end of the study. Faeces and other remains were removed by siphoning every morning at 9.30 am before the commencement of feeding. Throughout the experiment, each aquarium was partially (1/3 capacity of water) cleaned daily, and the water was completely changed three times a week. Every two weeks, fish were weighed and the experimental rations were adjusted accordingly. The set-up was placed under the natural day-length conditions, i.e. between 11 and 12 hours of light. Water temperature was recorded once daily at 10 am. 6 days a week. Weekly water analyses were carried out to determine pH values using pH meter, and salinity values using salinometer. At the end of the experiment, fish in each aquarium were individually weighed, then were sacrificed and frozen at – 20ºC for final body composition analyses. Initial body analyses were performed on a pooled sample of 25 fingerlings, which were weighed and frozen prior to the study. Proximate analyses of water, protein, lipid and ash in body fish carcass were performed according to standard AOAC (1990) methods. Body weight gain BWG (g/fish), specific growth rate SGR, (%/g/day), food conversion ratio FCR, protein efficiency ratio PER, protein productive value PPV, energy utilization EU and survival rate SR, (%) were computed according to Jauncey and Ross (1982). Statistical analyses of experimental data were treated with one-way analysis of variance (ANOVA) and means were compared using Duncan's multiple range test (1955) (P > 0.05).

Table 1.Composition and proximate analyses (%) of experimental diets.

Item	Diet			
	1(CTR)	2	3	4
Feed Ingredient (% DM)				
Imported Fishmeal (62.6% P; 4.6% L)	61	50	40	30
Soybean meal (Solvent-extracted)	25	30	30	30
Wheat bran	5	8	14	18
Yellow corn	4	4	6	10
Linseed oil	3	6	8	10
Vitamin mixture [1]	1	1	1	1
Mineral mixture [2]	1	1	1	1
Proximate analyses(%DM)				
Dry matter	91. 60	89. 86	87. 64	86. 82
Crude protein (CP)	50. 05	45. 54	40. 46	35. 41
Crude lipid (CL)	5. 60	10. 06	12. 14	13..98
Crude fiber (CF)	2. 49	3. 05	3. 54	4. 14
Ash	16. 21	13. 90	11. 16	10. 04
Nitrogen free extract (NFE)*	25. 65	27. 45	32. 70	36. 43
Gross energy (Kcal/100g) [3]	441.07	464. 63	477.19	481.41
Protein energy ratio (P/E mg P/ Kcal GE)	113.47	98. 01	84. 79	73..55

[1]- Vitamin mixture / kg premix containing the following: 3300IU vitamin A, vitamin D, 410IU vitamin E, 2660mg vitamin B^1 , 133 mg vitaminB^2, 580mg vitamin B^6, 410 mg vitamin B, ^{12} 50 mg biotin,9330mg colin chloride, 4000mg vitamin C, 2660 mg Inositol, 330mg para- amino benzoic acid, 9330mg niacin, 26.60mg pantothenic acid.

[2]- Mineral mixture / k premix containing the following: 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine, 5 mg Cobalt.

[3]- Gross energy (GE kcal/100g diet) calculated as, 5.65, 9.45, and 4.2 kcal/g diet of protein, fat and carbohydrate, respectively, NRC (1993)

* Calc. By difference.

RESULTS AND DISCUSSION

No differences were found in water quality variables for aquaria of the four treatments. Water temperature throughout the experiment was 26.6 ± 0.4 ^{o} C and pH was 8.2 ± 0.03, while water salinity 35 ppt.

Table (1) shows feed ingredient and proximate analyses (%) of experimental diets. Crude lipid (CL) increased in the tested diets from 3 to 5.6 in D1; 6 to 10.06 in D2; 8 to 12.14 in D3 and from 10 to 13.98 in D4 respectively, so the ingredient contain additional crude lipid. Crude fiber increased from 2.49 in D1 to 4.14 in D4, while ash content decreased from 16.21 in Diet 1 to 10.04 in Diet 4. Gross energy (Kcal/ 100g) increased in tested diets when increased crude lipid and nitrogen free extract.

Results of the present work have shown that growth performance indices of fish fed diet 1 (50% P & 5.6% L) gave the best results. When protein level decreased and lipid increased in diet 2 & 3 (45%P &10% L & 40% P & 12% L respectively) nearly the same results, were obtained. The poorest growth rate was recorded when fish fed diet 4 of the least tested protein but highest lipid content (35% P & 14% L). This may indicate that excessive lipid may decrease feed intake restrict protein consumption and consequently retard growth (Kheir, 1997). On the other hand, it seems that 10% -12% dietary lipid level improved digestion and absorption of nutrients for seabream fish (Hassanen 1997). Kissil *et al*. (1983) estimated the dietary protein requirement of juvenile gilthead seabream reared in seawater to be approximately 40% of diet and 3800-kcal/ kg dry diet of metabolizable energy. Hassanen (1997) showed that the best growth of gilthead seabream (*Sparus aurata*) was obtained with diet of 38% protein and 10.36 lipid that contained 4370 -kcal/ kg dry diet than the diet contained 46% protein and 8.75% lipid (4353-kcal/ kg). The mean survival rate of fish fed at different test diets decreased from 100% to 90% for fish fed diet 4. (Table 2). No external abnormalities were observed among experimental fish.

As shown in table (3), feed intake decreased with decreasing dietary protein at all lipid levels. Statistical analysis among the four treatments showed no significant differences between feed intake by fish fed diet1 (50% P and 5.6 % L), diet 2 (45.54% P and 10 .0 % L) or diet 3 (40.46% P and 12.14 %L). Whereas fish fed diet 4, which contained 35.4% P and 13.98 % L showed the least feed intake among all. The best feed conversion ratio (FCR), protein utilization efficiency in terms of protein productive value (PPV) and protein efficiency ratio (PER) as well as energy utilization (EU) were achieved with diet 3 which contained (40.46% P and 12.14 % L). No significant differences between fish fed diet2, 3 or 4 in terms of protein utilization (PER, and PPV), on the other hand, there were no significant differences between fish fed diet1, 2& 3 in terms of energy utilization (EU).

Hassanen (1997) showed that when gilthead seabream (*Sparus aurata*) fed diet contained 38% P &10.5% L better feed conversion ratio, protein efficiency ratio and protein retention were obtained than fish fed diet contained 46% P & 8.7% L. Tabachek (1986) showed that PER was directly related to dietary lipid and inversely related to dietary protein of the Arctic charr *Salvelinus alpinus* . Hassanen (1987) mentioned that PER & PPV in a freshwater omnivore fish *Clarias lazera* was improved significantly when the protein level in the diet decreased. On the other hand, Hassanen (1988) reported that PER &PPV of *Tilapia nilotica* improved significantly as the lipid level in the diet increased. Kheir and Saad,(2003) determined the suitable lipid level which spares the dietary protein for maximum growth of three species *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Hypophthalmichthys molitrix*, found that a low level of protein 20% with a high level of lipid 6% had produced nearly equal growth and FCR similar to that with high protein 30% and low lipid 2%. They concluded that fish are able to store considerable quantities of lipid in their tissues to utilize fat as energy source in order to improve growth and feed utilization.

Table (2) Growth performance indices and survival rate of Sharp-snout Sea bream (*Diplodus puntazzo*) fingerlings fed different levels of dietary protein and lipids.

Diet *	Initial Weight IW (g/fish)	Final Weight FW (g/fish)	Indices			Survival Rate (SR) (%)
			Weight Gain (g/fish)	ADG [1] (mg/ fish/d)	SGR [2] (%/day)	
1	4.38	16. 05 [a]	11. 67 [a]	166.71 [a]	1. 86 [a]	100 [a]
2	4.40	15. 86 [a]	11. 46 [a]	163.71 [a]	1. 83 [a]	100 [a]
3	4.39	15. 64 [a]	11. 25 [a]	160.71 [a]	1. 82 [a]	100 [a]
4	4.40	13. 94 [b]	9. 54 [b]	136.29 [b]	1. 65 [b]	90 [b]
LSD** (P<0.05)	0.0	1. 54	1. 55	22. 14	0.151	7.61

* As defined in Table (1).
[1] ADG = Average daily gain = FW – IW g / experimental period (in days).
[2] SGR = Specific growth rate = ln final weight – ln initial weight / time (day) X 100
a,b,c: Means with different letters within the same column differ significantly (P< 0.05).
**Least significant differences.

Table (3) Feed and nutrient utilization by Sea bream (*Diplodus puntazzo*) fingerlings fed at different levels of protein and lipid in diets.

Diet	Feed utilization		Protein utilization		EU % [5]
	FI(g) [1]	FCR [2]	PER [3]	PPV % [4]	
1	24.77 [a]	2.12 [ab]	.094 [b]	20.76 [b]	21.18 [a]
2	23.66 [a]	2.06 [ab]	1.06 [ab]	23.02 [ab]	20.75 [a]
3	22.98 [ab]	2.04 [b]	1.21 [a]	25.84 [a]	20.88 [a]
4	21.49 [b]	2.25 [a]	1.25 [a]	26.37 [a]	18.95 [b]
LSD (P< 0.05)	2.18	0.15	0.23	4.15	1.61

[1] FI= Feed intake on dry matter basis.
[2] FCR (Food conversion ratio) = (g dry feed / g live body gain).
[3] PER (Protein efficiency ratio) = (g live body gain./ g protein intake).
[4]. PPV %(Protein productive value) = (g protein gain / g protein intake X100)
[5] EU % (Energy utilization) = (Energy gain in fish (kcal) / energy intake (kcal) X 100)
a,b,c: Means bearing different letters within the same column differ significantly (P< 0.05).

Table (4) shows carcass composition (%) on dry matter basis of seabream *Diplodus puntazzo* before and after feeding at different levels of protein and fat for 71 days. In general, results of the present study indicated that dry matter and protein content in carcass fish increased with increasing protein and decreasing lipid in diet. On the contrary, lipid content was increased with increasing the lipid level and decreasing protein level in the diet. Tibaldi *et al*. (1996) found an inverse relationship between water and lipid content and a positive correlation between dietary lipid and body lipid for juvenile dentex (*Dentex dentex*). Williams and Robinson (1988) reported that body lipid was increased with increased dietary lipid. Siddiqui *et al*. (1988) noticed that the body protein content increased with increasing the protein levels in the diet of *O. niloticus*. They also recorded that positive correlation between body lipid and dietary lipid may indicated that when dietary lipid was supplied in a high level, a proportion of this lipid was deposited in the alimentary canal of the studied fish. Tabachek (1986) stated that when dietary lipid was too high, the excess lipid was deposited within body tissues. Chen and Tsai (1994) found that increased dietary protein resulted in a decrease in body lipid content in juvenile grouper *Epinephelus malabaricus.* Landesman *et al*. (1985) have found no increase in body protein content for goby (*Poecilia reticulata*), but the apparent decrease in body protein of fish fed on high lipid diet may be due to a proportional decrease in the percentage of protein percent in tissue as a result of increased tissue lipid.

Both the feed cost and the feed conversion ratio determine the final cost of the fish production. The differences in feed conversion between test diets had resulted in a higher cost / kg fish produced with the highest protein diet. Table (5) showed that fish fed diets 2,3 or 4, contained 45%P, 40%P and 35% P were very profitable than the control diet (diet1) which contained the highest protein level (50%P). Decreasing protein level from 50% to 40% and increasing lipid from 5% to 12% in diet, has improve feed conversion ratio and lowered the cost of used ingredients. Therefore, the cost of production for one kg of fish decreased by 16.45% when diet 3 was used and by 15.8% when diet 4 was used as compared control diet 1.

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Table (4) Carcass composition (% dry matter) of Sea bream (*Diplodus puntazzo*) fingerlings at start and of feeding trial (71days).

Diet *	Dry matte (DM %)	% On Dry Matter Basis			Energy content (Kcal/100g) [1]
		Crude protein (CP %)	Crude lipid (CL %)	Ash (%)	
At start	23.60	59.49	12.26	24.62	451.98
At end 1	31.52 [a]	63.04 [a]	20.76 [b]	16.12 [a]	552.36 [b]
2	31.04 [ab]	62.92 [a]	21.80 [ab]	15.31 [ab]	561.51 [ab]
3	30.84 [ab]	62.60 [ab]	23.44 [a]	13.94 [ab]	575.20 [a]
4	30.14 [b]	62.48 [b]	24.20[a]	13.42 [a]	581.70 [a]
LSD (P < 0.05	0.91	0.42	2.48	1.97	21.05

[1] Energy content (Kcal/ 100g diet) calculated according to NRC (1993) using the following calorific values; 5.65, 9.45 and 4.2 Kcal /g whole body of protein, fat and carbohydrates, respectively.
a,b,c: Means bearing different letters within the same column differ significantly (P<0.05).
* As defined in Table (1).

Table (5) Cost of ingredients and profitability of tested diets fed to Sharp-snout Seabream (*Diplodus puntazzo*).

Ingredients	Price (LE/Kg)	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	6.5	3.965	3.25	2.600	1.950
Soybean meal	2.1	0.525	0.63	0.630	0.630
Wheat bran	0.75	0.038	0.06	0.105	0.135
Yellow corn	0.95	0.038	0.38	0.057	0.095
Linseed oil	10.00	0.300	0.60	0.800	1.000
Vitamin & Mineral mix.	12.00	0.240	0.240	0.240	0.240
<u>**Profitability**</u>					
Total cost of feed (EP)		5.11	5.16	4.432	4.05
FCR		2.12	2.06	2.04	2.25
Cost of 1 kg fish produced (EP)*		10.82	10.63	9.04	9.11
%Cost to control diet**		-	-1.76	-16.45	-15.80

*Cost / 1 kg fish produced = Cost / (kg feed X FCR)
**Cost of Diet 2 or 3 or 4 - Cost of Diet 1 / Cost of Diet 1 X 100
-Diet 1 , 50.05 % CP and 5.6 % L, Diet 2, 45.54% CP and 10.06 % L, Diet 3, 40.46 % CP and 12.14% L, Diet 4 , 35.41 % CP and 13.98 % L.

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