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Development of Diets for Gilthead Bream *Sparus aurata* L. Cultured in Egypt.

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ABSTRACT. Two novel raw materials, dry germinated soyabean meal and fish silage, were used as the major protein sources in *Sparus aurata* feeds. Amino acid analyses of both materials, on one hand and the experimental fish on the other hand, proved that they meet or exceed the requirements of the species.

Three balanced diets were formulated on the basic idea of replacement of fish meal either partially, by defatted soyabean meal/dried germinated soyameal, or completely by a mixture of both soyabean meal and fish silage. Preliminary observations, under aquarium conditions, indicated that these artificial feeds are appropriate for the species.

Introduction

There is ample potential in Egypt, both in environment and in the favorable climate, for the development of fish farming utilizing new technology already in use in Europe. Recent interest in marine fish farming in Egypt stems from 1976, when culture of gilthead bream *Sparus aurata* L. was initiated. Trials of weaning the species in brackish water ponds or cages were successful (Eisawy and Wassef, 1984 and Wassef and Abu El Wafaa 1985). As part of the ongoing program to develop commercial production techniques and to maximize the yield per unit area, a low cost diet is required. In many countries, high quality fish meal is the primary protein source of the feed in intensive culture systems. However, alternative sources of good quality, but

*Present address, Girls College, Jeddah, Saudi Arabia less expensive, proteins have been investigated in many countries as well as in Egypt. Soyabean meal was chosen as one of the dietary ingredients as shown by the preliminary results of the first trial to feed gilthead bream artificially in Egypt (Wassef and Abu El Wafaa 1985). It is well documented that anti-nutritional factors, such as protease (trypsin) inhibitors, are present in soyabeans (Finney 1982). These factors are, in part, responsible for decreased activities of digestive proteolytic enzymes and for reduced growth when raw soyabeans were fed to fish (Viola *et al.* 1983). Controlled processing and treatment of the meal prior to inclusion is therefore a must. In this context, means of reducing the effects of protease inhibitors in soyabeans, thereby increasing the susceptibility of soya proteins to the action of protease enzymes, were recently established (Wassef *et al.* 1988). But because of sea bream intolerance of carbohydrate, a whole soya product is more undesirable. The fish must additionally have a source of animal protein and this is to be provided from fish silage, which can be produced locally by making better use of waste fish (post-harvest fishery losses are about 20-30%), fish processing by-products and under utilized fisheries.

The objectives of the present work are formulation and preparation of a series of practical diets from soyabean meal and fish silage which fulfill the nutritional requirements of gilthead bream.

The technology and production process could also be applied, not only to sea breams, but also to other species such as sea bass, grey mullets and sole. It is hoped that the present study provides a further step in the development of sea bream culture.

Material and Methods

1. Soya Processing

Soyabeans were purchased locally in Alexandria. The seeds were germinated for 7 days, as previously shown by the author that germination and defatting reduced protease (trypsin) inhibitor activities (Wassef *et al.* 1988). Soyabeans were grown on plastic trays lined with wet cloth towels, covered to reduce evaporation, and placed into a glass plant-house at about 20°C. Fifty percent moisture is necessary for germination. Excessive moisture is unfavorable. Germination of the seeds can be accelerated by impaction, soaking in water and growth at a temperature of 30°C. However, a combination of low temperature and high moisture may favor development of certain fungi and bacteria detrimental to seedling growth (Finney 1982).

Germinated seedlings were dried in a ventilated oven at 50°C overnight and then milled using setting 2 of a Miag Mill, which gave the most uniform size particles in the range 0.18-1.0 mm. Soya flour was then sieved through a 0.71 mm hole-diameter sieve. On the other hand, defatted soyabean meal (hexane-extracted), and soya oil as well, were supplied by "Oils Processing Company" at Damanhour, (price is £E 750 per tonne). The average particle size of defatted soyabeans = 2 mm and typical composition would be as follows: crude protein 44-46%; oil 1-3%; moisture 12%; crude fiber 3.5-4%; ash 4-5%.

2. *Preparation of Fish Silage*

Acid preserved silage was produced in the laboratory, from freshly caught pelagic trash fish (other species available could also be used as well) adopting the method of Jackson *et al.* (1984). Nutritional characteristics of silage were also determined (Table 1).

TABLE 1. Composition (% dry weight) of raw materials.

Ingredients		Crude protein	Ether extract	Carbohydrates*	Metabolic energy	Calcium	Phosphorus
Fish silage	(FS)	73.4	17.1	1.2	14.7**	1.0	1.5
Soyabean meal, defatted	(SBM)	44.0	1.0	39.3	9.4	0.3	0.6
Fish meal	(FM)	65.0	4.0	5.0	11.8	6.0	3.0
Dried germinated soya	(DGS)	43.9	16.7	28.2	15.4**	0.2	0.6
Cod liver-oil	(CLO)	–	100	–	35.0	–	–
Soyabean oil	(SBO)	–	100	–	37.0	–	–
Wheat starch	(WHST)	–	–	100	13.0	–	–
Calcium carbonate	($CaCO_3$)	–	–	–	–	38.0	–

*Includes nitrogen free extract and crude fiber.
**Estimated.

3. *Other Ingredients*

3.1 *Fish Meal*

Fish meal available in Egypt, is of variable quality and prices. Imported fish meal is the highest quality and is correspondingly expensive (£E 1200-1400 per tonne). Typical composition would be 70% protein and 9% fat. Indigenous fish meal are produced from a number of sources. In present work, fish meal (composition listed in Table 1) was provided from the principal fish meal factory at Aswan at a price of £E 800 per tonne.

3.2 *Vitamin Premix*

There is a lack of suitable vitamin or mineral premix for fish diets in local markets. At present, poultry premixes are used, often at very low levels. But, due to the differing requirements of poultry and fish, the vitamins are incorrectly balanced. In view of the high prices (£E 15 per kilogram) and inadequate inclusion of premixes, they are considered in the present work as unnecessary expense particularly when the fish are farmed in semi-intensive systems.

3.3 *Treatment*

Other ingredients were obtained from normal commercial sources at reasonable prices except for cod liver oil which was relatively expensive (£E 30/kg).

All dry feed ingredients were milled and sieved, as described for soybeans, to get the requisite particle size before inclusion.

4. Diet Preparation

Three diets were prepared from the ingredients listed in Table 1. The factor which determined maximum inclusion rate of fish silage was its high moisture content. The inclusion level should give a 1:1 ratio in the soyabean meal/fish silage mixture (on dry weight basis).

All ingredients, of each diet, were mixed well and then compressed by a laboratory scale California pelleting machine (CPM). The pellets were air dried for 4 hours before being packed.

5. Analytical Procedures

Proximate analysis was completed on all ingredients and the three test diets. Moisture, lipid, ash and Kjeldahl nitrogen were all determined as in AOAC (1980) methodology. Samples of experimental fish, *Sparus aurata*, and dried germinated soyameal (only) were subjected to amino acids analysis. Amino acid profiles were obtained following acid hydrolysis (24 h in 6*N* HCl at 110°C) under nitrogen and chromatographic separation on an amino acid analyser. Tryptophan was measured separately according to Matheson (1974).

6. Experimental Fish

Fry and young gilthead breams were obtained from the coastal waters at Rattama (about 220 km east of Alexandria). Methods of collection and transportation were previously mentioned (Eisawy and Wassef 1984). Total length varied from 2.2 to 5.3 cm; mean length 3.1 cm, and weight from 0.38 to 1.0 g; mean weight = 0.65 g.

The experimental system consisted of 9 rectangular glass tanks (3 replicates/treatment) of 230 L capacity, each filled with sea water 35 ppt salinity). Forty fish were kept in each tank for 41 days at ambient temperature that varied from 18 to 27°C.

The food (fry coarse pellets, 1.75 mm and 1.25 g each) was supplied by hand as long as fish took it, twice a day, six times a week. Fish were weighed wet in preweighed sea water containers.

Results and Discussion

1. Nutritional Requirements of *Sparus aurata*

1.1 Total Protein

According to the literature this should be at least 40% of dry diet (Sabaut and Luquet 1973, Kissil *et al.* 1982).

1.2 Amino Acids (AA)

Nineteen amino acids were determined, in the present work, for young sea breams, germinated soya meal and listed together with those of fish silage in Table 2 (Sabaut and Luquet, 1974, loc. cited, Wilson, 1985). Only four AA's estimated to be needed by the species (Table 2). It is obvious that, both ingredients (soya meal and fish silage) meet or exceed the essential amino acid pattern for sea bream. The first limiting amino acid is methionine, then tryptophan (Table 2).

TABLE 2. Amino acid profiles for soyabean meal, fish silage and sea bream muscles.

Amino acid	Dry germinated soyabeans (g/100 g)	Fish* silage	Sea bream muscles (g/100 g protein)	Requirements***
Arginine	5.59	4.97	4.14	1.7/34**
Histidine	4.30	3.24	1.97	–
Threonine	2.97	3.58	5.63	–
Isoleucine	3.64	3.65	2.18	–
Leucine	6.09	6.08	6.06	–
Valine	3.86	4.13	3.60	–
Lysine (LYS)	4.49	7.23	6.49	1.7/34
Methionine	1.25	2.48	1.88	1.4/34
Tryptophan	–	0.87	–	0.2/34
Phenyl alanine	4.30	3.24	1.75	–
Aspartic acid	15.20	8.19	8.85	–
Serine	4.15	3.60	6.63	–
Glutamic acid	13.03	12.09	11.54	–
Glycine	3.14	4.80	17.45	–
Alanine	3.54	5.13	14.57	–
Tyrosine	4.03	2.80	1.22	–
Proline	4.46	3.57	3.71	–
Cystine	1.13	0.67	–	–
Availability LYS	4.31	–	–	–
% availability	96%	100%	–	–
% recovery	83%	79%	–	–

*Wassef, 1990.
**Percent of protein in the diet.
***After Sabaut and Luquet, 1974 (Loc. cited Wilson, 1985).

Yone (1975) measured the requirement for the Japanese red sea bream *Chrysophrys major* for 10 essential amino acids. However, the relation between gilthead bream and red sea bream is still uncertain.

1.3 Carbohydrates

Not more than 10% of diet, since species has low capability of carbohydrate assimilation (Marias and Kissil 1979).

1.4 Lipids and Essential Fatty Acids

The use of vegetable oil alone and an excessive amount of oil in the diet is undesirable for sea bream. The superior performance of fish oil over soyabean oil is supported by evidence, in the literature, indicating better growth of certain fish, especially marine species, when a source of marine oil is used in the diet (Kissil *et al.* 1982).

Eight percent as a mixture of soyabean oil (5.7%) and cod liver oil (2.3%), (Sabaut and Luquet 1973). Ten percent capelin oil for juveniles and 5% for ongrowing stage (Kissil and Gropp 1984).

The most effective essential fatty acids (EFA) are 20:5 w3 and 22:5 w3 (Koven and Kissil 1984).

1.5 Vitamins

Quantitative requirements of the fish were determined for only: B6 to be 1.97 mg/kg dry diet (Kissil *et al.* 1981).

1.6 Energy

Level between 19610-19633 J/g (Marias and Kissil 1979). The combination of 5% fish oil added to 40% diet represents a protein ratio of 105 mg protein/K cal or 3800 kcal/kg diet of gross available energy (Kissil and Gropp 1984).

2. Diet Formulations

Three balanced diets were formulated to the nutritional constraints indicated above (Table 3), and the composition of each was estimated (Table 4).

TABLE 3. Composition of three diet formulae for gilthead bream *Sparus aurata*.

Ingredients	% inclusion		
	Diet I (SBM/FM)	Diet II (SBM/FS)	Diet III (DGS/FM)
Fish meal	24.2	–	24.2
Soyabean meal	66.5	45.0	–
Fish silage/soyabean meal	–	42.9	–
Dry germinated soya	–	–	66.7
Cod liver oil	2.7	–	2.7
Soyabean oil	6.6	5.6	–
Wheat starch	–	4.7	–
Calcium carbonate	–	1.8	–
Cellulose	–	–	6.4

TABLE 4. Composition (% dry weight) of the three diets.

Analysis	Diet I (SBM/FM) %	Diet II (SBM/FS)	Diet III (DGS/FM)
Crude protein	45.0 (15.7)*	45.0 (15.7)	45.0 (15.7)
Ether extract	11.0 (3.7)	10.0 (3.7)	14.8 (3.7)
Carbohydrates	27.3	29.1	20.0
Metabolic energy (mJ/Kg dry matter)	12.5	12.0	14.1
Calcium	1.6	1.01	1.5
Phosphorus	1.1	0.73	1.1

*Percentage that should be of marine origin.

3. Preliminary Feeding Trial

Prior to the start of feeding experiment, young sea breams underwent a 3-week conditioning period during which they readily adjusted to diets and standardized environmental conditions. Unfortunately, the feeding trial lasted only for 20 days further, and terminated after the electricity cut-off the system and the consequent death of all experimental fish. However, preliminary results obtained so far (Table 5) indicated a satisfactory growth rate as compared to that obtained for wild young fish (Eisawy and Wassef 1984). Percentage mortality ranged from 5 to 7% throughout the whole experimental period. But these results are not sufficient to elucidate a precise comparison between the three diets. Next paper will overcome this problem.

TABLE 5. Growth characteristics of *Sparus aurata* on the three diets.

Diet*	Mean Fish Weight (g)				
	Initial day (0)	Final day (21)	% gain / day	day (41)	% gain / day
Diet I	0.65 ± 0.4	1.63 ± 0.6	0.05	8.39 ± 0.5	0.34
Diet II	0.65 ± 0.4	2.00 ± 0.5	0.06	11.15 ± 0.4	0.46
Diet III	0.65 ± 0.4	1.85 ± 0.4	0.06	10.10 ± 0.7	0.41

* ± Standard deviation.

The present work's experimental facilities (closed system) failed to prove convenient for sea breams, instead, they can be kept for about 8 months in an open circulation system (constant water flow) with no mortality at all (Kraljevic 1984).

Numerous workers have studied the effects of artificial diets on the growth of gilthead bream, (Marias and Kissil 1979, Ramos and Kobayashi 1981, Kissil *et al.* 1981, 82, Kraljevic 1984, and Divanach *et al.* 1986). However, their data were mainly based on either purified or semi-purified test diets. Although their results provided a significant ground for studies of sea bream nutrition, this problem requires further investigations particularly under local situations in Egypt.

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References

AOAC (1980) *Official Methods of Analysis*, 13*th* ed., Assoc. Official. Agric. Chemist., Washington, D.C. 1018 p.

Divanach, P., Kentouri, M. and **Dewavrin, G.** (1986) Sur le sevrage et l'evolution des performances biologiques d'alevins de daurades, *Sparus auratus*, provenant d'elevage extensif, apres remplacement des nourrisseurs en continu par des distributeurs libre service, *Aquaculture* **52:** 21-29.

Eisawy, A. and **Wassef, E.** (1984) Preliminary studies on rearing of the gilthead sea bream, *Sparus aurata*, in brackish water ponds, *Aquaculture* **38:** 255-260.

Finney, P.L. (1982) Effect of germination on cereal and legume nutrient changes and food or feed value: a comprehensive review, *In:* **Nozzolillo, C., Lea, P.J.** and **Loewus, F.A. (ed.)**, Mobilization of Reserves in Germination, *Recent Advances in Phytochemistry*, Plenum Press, New York **17:** 229-305.

Jackson, A., Kerr, A. and **Cowey, C.** (1984) Fish silage as a dietary ingredient for salmon. I. Nutritional characteristics, *Aquaculture* **38:** 211-220.

Kissil, G. Wm., Cowey, C., Adron, J. and **Richards, R.** (1981) Pyridoxine requirements of the gilthead bream *Sparus aurata, Aquaculture* **23:** 243-255.

Kissil, G. Wm., Meyers, S., Stickney, R. and **Gropp, J.** (1982) Protein energy ratios in the feed of the gilthead bream *Sparus aurata, Proc. Warm Water Fish Cul. Workshop World Mar. Soc. Sp. Publ.* **3:** 145-152.

Kissil, G. Wm. and **Gropp, J.** (1984) Optimal protein energy ratios in gilthead bream (*Sparus aurata*). *in:* **Rosenthal, H.** and **Sarig, S. (ed.)**, *Feeds Res. Aq. Proc. 2nd Seminar, 1984, Hamburg. E.M.S. Sp. Publ.* **8:** 99-101.

Koven, W. and **Kissil, G. Wm.** (1984) Requirements for w3 polyunsaturated fatty acids in the gilthead sea bream (*Sparus aurata*). *in:* **Rosenthal, H.** and **Sarig, S. (ed.)**, *Feeds Res. Aq. Proc. 2nd Seminar, 1984, Hamburg. E.M.S. Sp. Publ.* **8:** 93-97.

Kraljevic, M. (1984) On the experimental feeding of sea bream (*Sparus aurata* L.) under aquarium conditions, *Acta Adriat.* **25** (1/2): 183-204.

Marias, J. and **Kissil, G. Wm.** (1979) The influence of energy level on the feed intake, growth, food conversion and body composition of *Sparus aurata, Aquaculture* **17:** 203-219.

Matheson, N.A. (1974) The determination of tryptophan in purified protein and in feeding stuffs, *Br. J. Nutr.* **31:** 393-401.

Ramos, J. and **Kobayashi, K.** (1981) Influence of different diets on the growth of gilthead bream *Sparus aurata, Inf. Tec. Inst. Invest. Pesq.* **82:** 1-15.

Sabaut, J. and **Luquet, P.** (1974) Nutritional requirements of the gilthead bream *Chrysophrys aurata*. Quantitative protein requirements, *Mar. Biol.* **18:** 50-54.

Viola, S., Mokady, S. and **Arieli, Y.** (1983) Effects of soyabean processing method on the growth of carp (*Cyprinus carpio*), *Aquaculture* **32:** 27-38.

Wassef, E. and **Abu El Wafaa, M.** (1985) Effect of various dietary proteins on the growth and body composition of gilthead bream *Sparus aurata, J. Egypt Vet. Med. Ass.* **45** (1): 41-52.

Wassef, E., Palmer, G. and **Poxton, M.** (1988) Protease digestion of the meals of ungerminated and germinated soyabeans, *J. Food Sc. Agric.* **44:** 201-214.

Wassef, E.A. (1990) Experiments on nutritional properties and storage of fish silage, *Com. Sci. & Res. Alex.* **31:** 143-160.

Wilson, R.P. (1985) Amino acid and protein requirements of fish, *in.* **Cowey, C.B., Mackie, A.M.** and **Bell, J. (eds)**, *Nutrition and Feeding in Fish*, Academic Press, London, pp. 1-16.

Yone, Y. (1975) Nutritional studies of red sea bream *Chrysophrys major, Proc. 1st. Int. Conf. Aquacul. Nut.* **Oct. 1975:** 39-46. Lewes/Rehoboth, Delaware, USA.

إعـداد عـلائق صناعيـة لتغـذيـة أسماك الدنيس المستـزرعـة في مصر

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المستخلص . يعتبر مجال التغذية الصناعية لأسماك المزارع (وخاصة الأنواع البحرية منها) من المجـالات الهامة والحديثة للبحث العلمي في مصر . ويهدف إلى التوصل إلى إعداد وجبات صناعية متزنة لتغذية الأسماك المرباة ، وذلك لزيادة معدلات النمو وبالتالي الحصول على محصول سمكي أوفر . ويراعي عند اختيار المواد الخام المستخدمة في هذه العلائق أن تكـون متوافرة في السوق المحلي وبسعر رخيص حتى تنخفض تكاليف التصنيع وبالتالي يسهل توافرها على المستوى التجاري فيما بعد .

وفي هذا البحث تم اختيار كل من مسحوق فول الصويا بعد إنباته (Germinated Soyabean Meal) وسيلاج الأسماك (Fish Silage) كمصدرين رئيسين للمواد البروتينية في إعـداد علائق لتغذية صغار أسماك الدنيس البحرية . حيث أثبتت التحاليل الكمية للأحماض الأمينية لكل من هاتين المادتين صلاحيتهما لهذا الغرض وذلك لاحتوائهما على الكميات (من الأحماض الأمينية الأساسية) التي تفي بالمتطلبات الغذائية للأسماك التي تحت الاختبار .

وقد تم إعداد ثلاث وجبات صناعية متزنة على هيئة كبسولات غذائية (Pellets) يتم بوساطتها إحلال كل من مسحوق فول الصويا أو سيلاج الأسماك إحلالاً جزئيًا أو كليًا محل دقيق السمـك (Fish Meal) المرتفع الثمن نسبيا ، والتي يجري حاليا استخدامه لمعظم الأسماك المستزرعة .

وقد أثبتت التجارب المبدئية لتغذية صغار أسماك الدنيس (في أحواض زجاجيه معملية) على تلك الكبسولات معدلات نمو لا بأس بها مقارنة بمعدلات النمو لمثيلاتها في البيئة الطبيعية .

* العنوان الحالي : كليـة التربيـة للبنات بجـــدة ، الرئاسـة العامـة لتعليم البنات ، المملكة العربية السعودية .