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EVALUATION OF ANIMAL AND PLANT BY-PRODUCTS AS CONSTITUENTS IN DIETS FOR SEABREAM SPARUS AURATA L.

By

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Thesis submitted in partial fulfilment of the requirements of the Council for National Academic Awards for the degree of Doctor of Philosophy.

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In Collaboration with
National Marine Research Centre, Athens

August 1991

To my parents, for all their unbounding encouragement and support throughout my education.

Nengas, Ioannis., 1991.

Evaluation of animal and plant by-products as constituents in diets for seabream Sparus aurata.

Abstract

The principle aim of the study was to evaluate the nutritional value of animal and plant by-products, with the objective of improving the cost effectiveness of diets for culturing seabream (Sparus aurata). The programme of work was initially directed towards applying the current methodology used in fish nutrition research to establish reliable digestibility coefficients for various feed ingredients. A selection of animal and plant materials were tested for digestibility within a reference basal diet designed for seabream. Ingredients for special consideration included poultry by-product meal, feather meal, meat and bone meal, solvent extracted and full fat soyabean meal as well as various other plant derived materials. Comparative values for protein, energy and lipid digestibility coefficients were assessed. On the basis of these measurements, experiments were undertaken to evaluate the optimum inclusion levels of promising protein and energy sources as a replacement for the fishmeal component in diets for seabream. Emphasis was made on recent advances in feed processing technology. Raw materials were evaluated in terms of proximate analysis, amino acid profiles and the degree of heat treatment effects on protein quality and availability. Several indices relating to these included lysine availability, cresol red values and trypsin inhibitor levels for soya products. Growth performance and feed utilization trials were conducted mainly on juvenile fish in recirculation systems under controlled conditions of temperature and salinity. Nutritional parameters such as specific growth rate (SGR), feed efficiency (FE) and protein utilization parameters were determined in each successive trial. The results were favourable with respect to the partial inclusion of animal products in seabream diets. Poultry byproduct meals proved encouraging even at high inclusion levels. The growth performance of fish fed soyabean meal and full fat soya were initially favourable and merited further consideration. These preliminary trials led to a series of practical diet formulations to contain multi-ingredient components, which confirmed previous findings. Variable results, however, were reported for full fat soyabean meal in this experiment. Finally, the programme of research was presented in the context of the rapidly expanding mariculture industries of southern Europe with a particular relevance to Greece.

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CHAPTER 1

1.1 GENERAL INTRODUCTION

1.1.1. The status of European Aquaculture

The European region is the second largest aquacultural producing area, representing about 16% of the world farmed fish production (Kaushik, 1989a).

Salmonids represent the major group of fresh water finfish cultivated in most countries of Europe, primarily represented by the rainbow trout (Oncorhynchus mykiss) (OECD, 1986). Figure 1, presents some recent data on freshwater finfish production in different European countries.

Inland pond fish production in western Europe is remarkably lower than that of the eastern European countries. Considerable effort is being made towards the intensification of the existing extensive culture of several pond fishes such as the common carp, tench, roach and to a smaller extent pike. Interest has also being developed for the intensive or semi intensive culture of new species like the sturgeon (Acipenser guildenstsdti), tilapia (Oreochromis spp) and catfish (Ictalurus punctatus) given their excellent growth rates (Hilge and Arrington, 1989).

The production of finfish in the seawater environment is concentrated mainly in Scandinavia and in the U.K. (OECD,

1986). The four major groups that can be identified as having further potential with respect to their culture are salmonids, turbot (Scopthalmus maximus), seabass (Dicentrarchus labrax) and seabream (Sparus aurata) (Quevellou and Bories, 1989; Fermandes, 1989). Figure 2 details recent information on marine finfish production in different European countries.

Currently, the gilthead seabream is mainly cultivated in Spain (100 tonnes), Greece (250 tonnes) and Turkey. An increase in production is, however, expected to grow at least five fold within the next five years among the Mediterranean countries as a whole (White, 1986; Kallifedas, 1991).

The production of shellfish is economically the biggest aquacultural activity in almost all the foresaid countries. Figure 3, shows production levels of mussels and oysters in Europe.

Cultivation of freshwater or marine crustaceans is currently very low. Fresh water prawn (Macrobrachium spp) production is estimated to be around 200 tons and few attempts are made to produce marine prawns resulting in a total production of only 80 tonnes for all countries (Kaushik, 1989a).

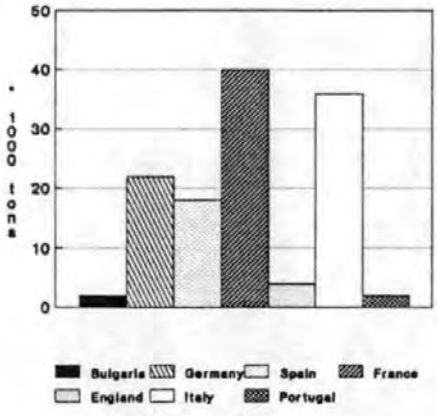

Figure 1.Freshwater finfish production in different European states.

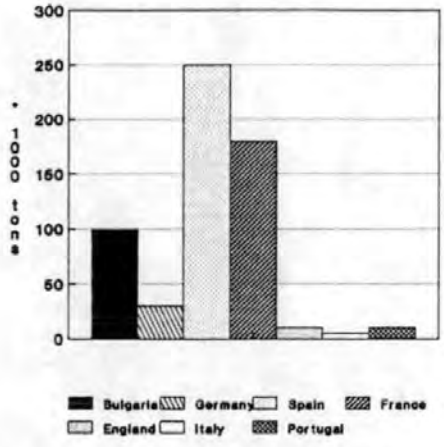

Figure 3. Shellfish production in different European states.

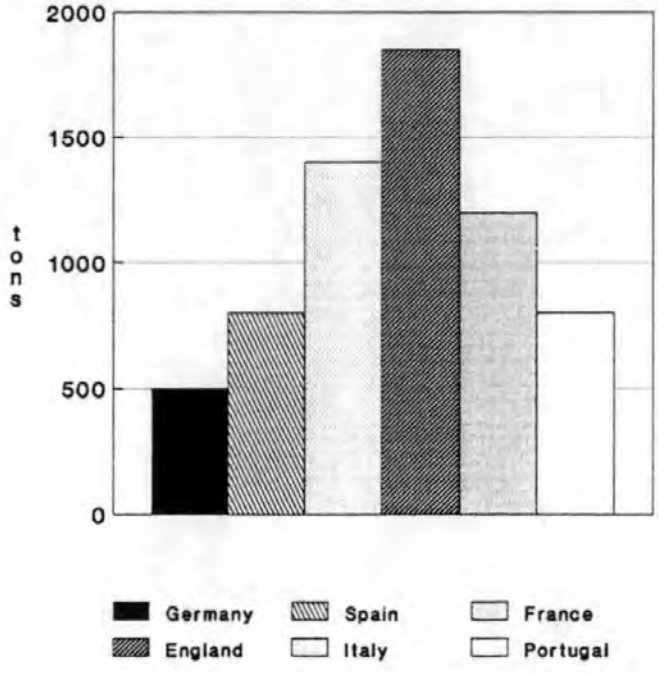

Figure 2. Marine finfish production in different European states.

Kaushik, 1989a

1.1.2. The situation of marine farm operation in Greece. Present perspectives and future outlook.

The ideal climatological conditions of Greece, the coastline (15,000 Km), the extended areas of lagoons, lakes and rivers, reduction of catches (landings), and the ever increasing demand for fish products has led to a rapid increase in aquaculture activities. Development has also been enhanced by the favourable financial motivations by EEC and the Greek government (Kallifedas, 1991).

Hence today there are almost 260 operational aquaculture units, rearing trout, salmon, carp, eel, tilapia, sea bream, sea bass and bivalves (Table 1) (Argirou, 1991).

The cultivation of marine fish is an important factor for the development of the local economy due to the high prices they attain, (£11/Kg), and the suitability of their culture to the Greek environment.

Sea bream (Sparus aurata) (Plate 1) and sea bass (Dicentrarchus labrax) are the main marine species cultured in Greece, and this is undertaken in the majority of cases intensively, in floating sea cages (Plate 2) but also extensively in lagoons and occasionally in earthern tanks (Claoudatos and Apostolopoulos, 1984). The fish farming operations depended largely on hatchery produced fry and juvenile fish imported form Cyprus, France, Turkey, Italy, Spain and Yugoslavia. Since 1984, however, fry production for both bass and bream started to rise steadily to levels

Table 1. Recent aquaculture production in Greece (Argirou, 1991).

	1986		1989	
Species	Quantities in tonnes	%	Quantities in tonnes	%
Trout	1800	37.50	2250	35.50
Carp	100	2.00	300	4.70
Eel	6	0.10	15	0.20
Seabream/bass	90	1.90	600	9.20
Mytilus sp.	230	4.80	1000	17.30
Lagoons	2574	53.70	2400	37.80
Rest	-	-	80	0.30
Total	4800		6745	

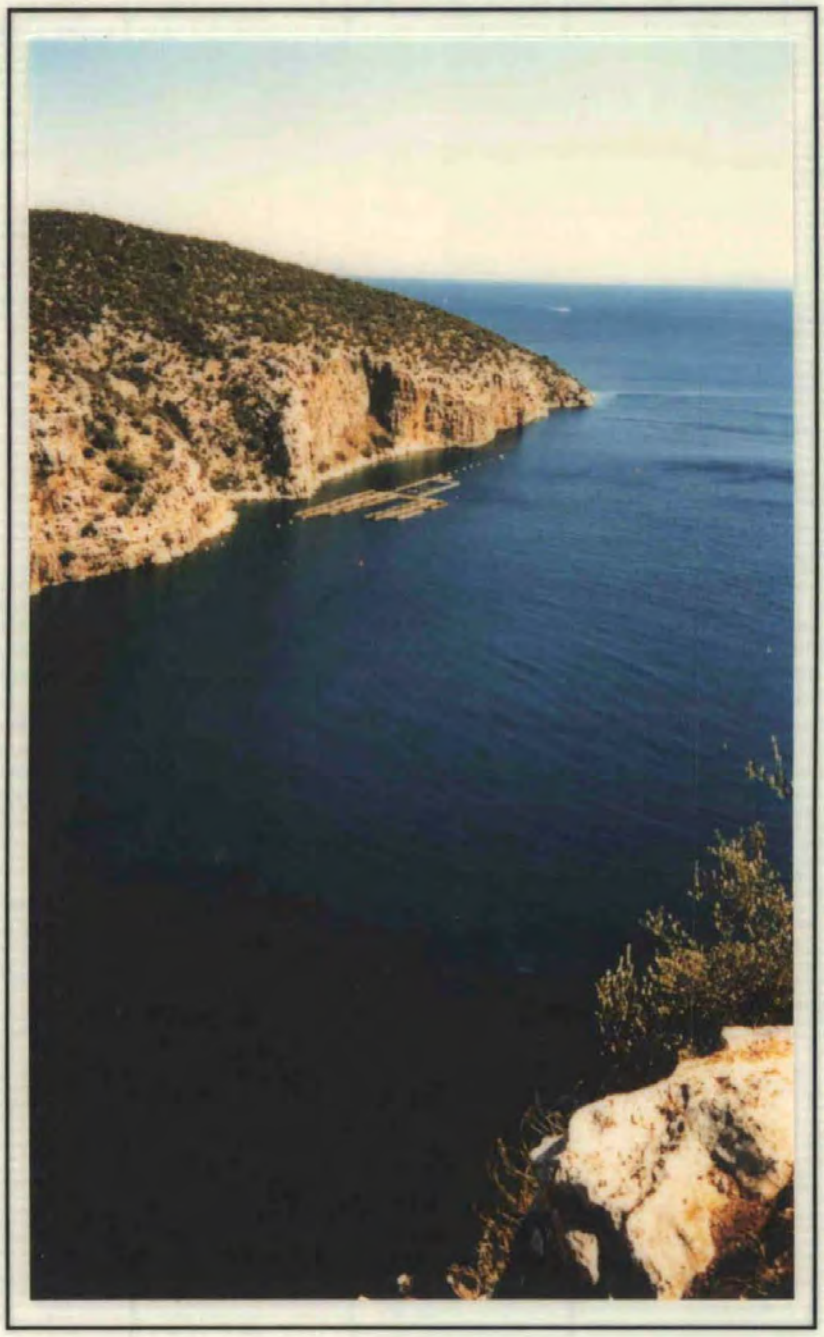

Plate 2. Typical cage site for the intensive rearing of seabream in Greece.

Plate 1. Production size seabream of approximately 500g body weight.

sufficient to support a considerable amount of sea cage rearing units. New techniques together with the use of nutrient enriched starter diets and improved husbandry methods have led to increased survival at the critical larval stages. This has greatly contributed to the potential expansion of this industry in Greece (Vlassopoulos, 1986; Claoudatos and Apostolopoulos, 1986).

However, despite the steady supply of fry, the production of marketable sized fish has not increased accordingly. A number of reasons are associated with this failure to achieve target production levels. The absence of a swim bladder and spinal deformities resulting in high mortalities (50-90%) at the later larval stages was a major cause that almost reduced bass and bream culture to complete closure (Argirou, 1991). New techniques mainly in the "hatchery" stages largely overcame these limitations. Hence in 1988/89 the first production of fish (95-100%) free from swim bladder syndrome and spinal deformities were supplied into the market. In 1989, a total of 65 operational marine farm units in Greece produced 600 tonnes of marketable fish; and the production is estimated to increase to approximately 5000 tonnes by 1994 (Claudatos, 1990).

Production costs are a major drawback for a marine fish farm unit. A typical budget can be separated according to fry purchase 40%, feeds 30% and labour, fixed costs, energy,

overheads e.t.c. 30% (Alexis et al., 1984). The price of the fry is fixed and determined by the hatcheries. Feed conversion ratios and composition of the diets are, however, areas open for research and improvement in order to minimize total production costs and to determine the commercial feasibility of marine fish farming. Feed conversion ratios of about 2.5:1 are a satisfactory value for sea bream and bass cultivation units today (Frentzos and Sweetman, 1989). These high values can be significantly improved considering that ratios less than 2:1 are quite achievable for salmonids during the later ongrowing stages (Crampton, 1987). The number and quantity of daily meals for different temperature regimes can also play a significant role in reducing such economically unacceptable values for bream. Therefore, optimum feeding schedules for this species requires more investigation.

Unlike most domesticated farm animals, the majority of fish species currently farmed including sea bass and sea bream are carnivorous, and consequently have a high dietary requirement for protein (Fowler and Banks, 1976). This has meant that the fish farmer has been faced with the problem of supplying fish with diets that must attain relatively high conversion rates and productivity. At present, good quality fish meals supply the major proportion of the protein component within commercial type fish feed diets (Watanabe

and Pongmaneerat, 1991). The average inclusion level of fish meal may vary on average between 25 and 65% (Tacon, 1981). Apart from being an expensive feed-stuff, quality fish meals of relatively constant nutritional value and composition are becoming progressively more difficult to find on a regular basis. Clearly, alternative and ideally, less expensive sources of good quality protein, in addition to fish meal must be found.

Unfortunately, in the majority of cases, the range of concentrate protein sources tested as substitutes for fish meal have not been successful in terms of their protein digestibility and biological value (BV) to fish when compared with good quality fish meal products (Mann, 1967; Van der Wind, 1973; Koops et al., 1976). Consequently protein rich ingredients such as animal byproducts, including meat and bone meals, hydrolysed feather meal , blood meal, poultry meat meals and plant byproducts, including soyabean meals, corn gluten meals, sunflower meal, to mention but a few, have normally been considered as secondary protein sources. In general, these protein sources constitute up to half the total protein component within many commercial fish feeds (Tacon, 1981). Despite, however, the wide variety of animal and plant ingredients which have been evaluated for fish, the selection of feedstuffs by the feed compounder is based on their relative cost, nutritive value, availability, and the ultimate market value of the farmed fish (Tacon, 1987).

As attempts are made to utilize greater amounts of novel proteins within compounded fish feeds, the problem of feed acceptability or palatability becomes greater. Exogenous dietary feeding stimulants or attractants together with new processing technologies are therefore a prerequisite before consideration is made for acceptance with certain species (Cho et al., 1985).

The development of a whole series of high quality protein sources, in addition to fish meal, will not necessarily reduce the cost of the finished feed to the fish farmer, but will, it is hoped, reduce the reliance of the fish feed manufacturing industry upon expensive fish meal based diets. Consequently the feed manufacturer operating into the next century will be able to ensure that the fish farming industry will receive a stable supply of relatively high quality feeds. These should not be subject to the shortcomings in the supply, quality and fluctuating cost of a single commodity such as fish meal.

Despite the fact that extended work has been carried out on the partial substitution of fish meal with other protein sources, attention has primarily been concentrated on fresh water fish, mainly salmonids including salmon and rainbow trout (Kaushik, 1989).

The expanding interest in marine fish farming and the need for less expensive feeds urgently calls for research on

the use of alternative protein sources both of animal and plant origin with specific relevance to Greece.

The scope of this work is to investigate the possibility of partially or even completely substituting animal and plant raw materials for fish meal in diets for the gilthead seabream (Sparus aurata). This species is now the major marine farmed fish produced in Greece with a projected expansion dependent on artificial feeds and compounded diets. The range of test ingredients included in the study are applicable to raw material availability in the southern Mediterranean region.

1.2. THE GILTHEAD SEABREAM (SPARUS AURATA L.)

In broad taxonomical terms, seabream belongs to the **Subphylum** of Vertebrata, **Class** of Osteicthyes, **Subdivision** of Teleostei, **Order** of Perciformes and the **Family** of Sparidae.

Its simplicity of adaptation to the production environment, both in artificial ponds and in lagoon environments, the relatively short time to attain commercial size and the quality of the flesh make the seabream a species much in demand from the growers in the Mediterranean region (Mazzola and Rallo, 1981). At the present, seabream is cultured intensively in floating cages rather than in ponds. Growth rate is not constant over the whole production period, but varies according to climate and especially in response to temperature (Claudatos and Appostolopoulos, 1986). Adult seabream are extremely hardy and resist temperature fluctuations (these range between 12^{o} and 25^{o} C over a typical Greek season), high ammonia and nitrite levels but are sensitive at low O_2 concentrations (<5mg/l) (Kentouri pers. com., 1991).

The species is commonly found in the Mediterranean region and only rarely in the Black sea. In the Atlantic ocean, it has a geographical distribution extending from the British Isles down to the Verde ls cape and the Canary islands (Whitehead et al., 1986). In Greece, it is located mainly in the Aegean Sea from Porto Lagos to the Dodecanease, within

the bays of Thermaico, Saronico, Amvracico, Patraico, and Corinthiaco and in the lagoon of Messologi (Papacostantinou, 1988) (Figure 4).

The fish may obtain a maximum length of 70 cm but is usually caught by fishermen at a typical length of 20 to 40 cm (350-500g). Seabream prefer to inhabit coastal waters and seldom move over wide areas. They usually feed singly or in small groups and are eurihaline in character, tolerating salinity levels from 5 ppt to 50 ppt and temperatures between 5^{o} to 35^{o} C (Olesen, 1986).

Juvenile individuals occupy depths of up to 30 m and the adults may be found even to 150 m.

Like many of the sparids, sea bream is a hermaphroditic species and a proportion of the all male population change to female after two years (Smart, 1988). The male fish, sexually matures at the age of two years old (20-30 cm, 300-400g) and the female at a slightly later period of two to three years (30-40 cm, 400-500g). The reproductive period usually extends from October to December (Whitehead et al., 1986), and this coincides with a fall in sea temperature throughout the region.

Their body is oval-shaped, laterally compressed and deep in front. The head is strong with a blunt snout and thickened lips. The jaws bear anteriorly six strong canines (long, curved and conical teeth) and laterally, four to five rows of

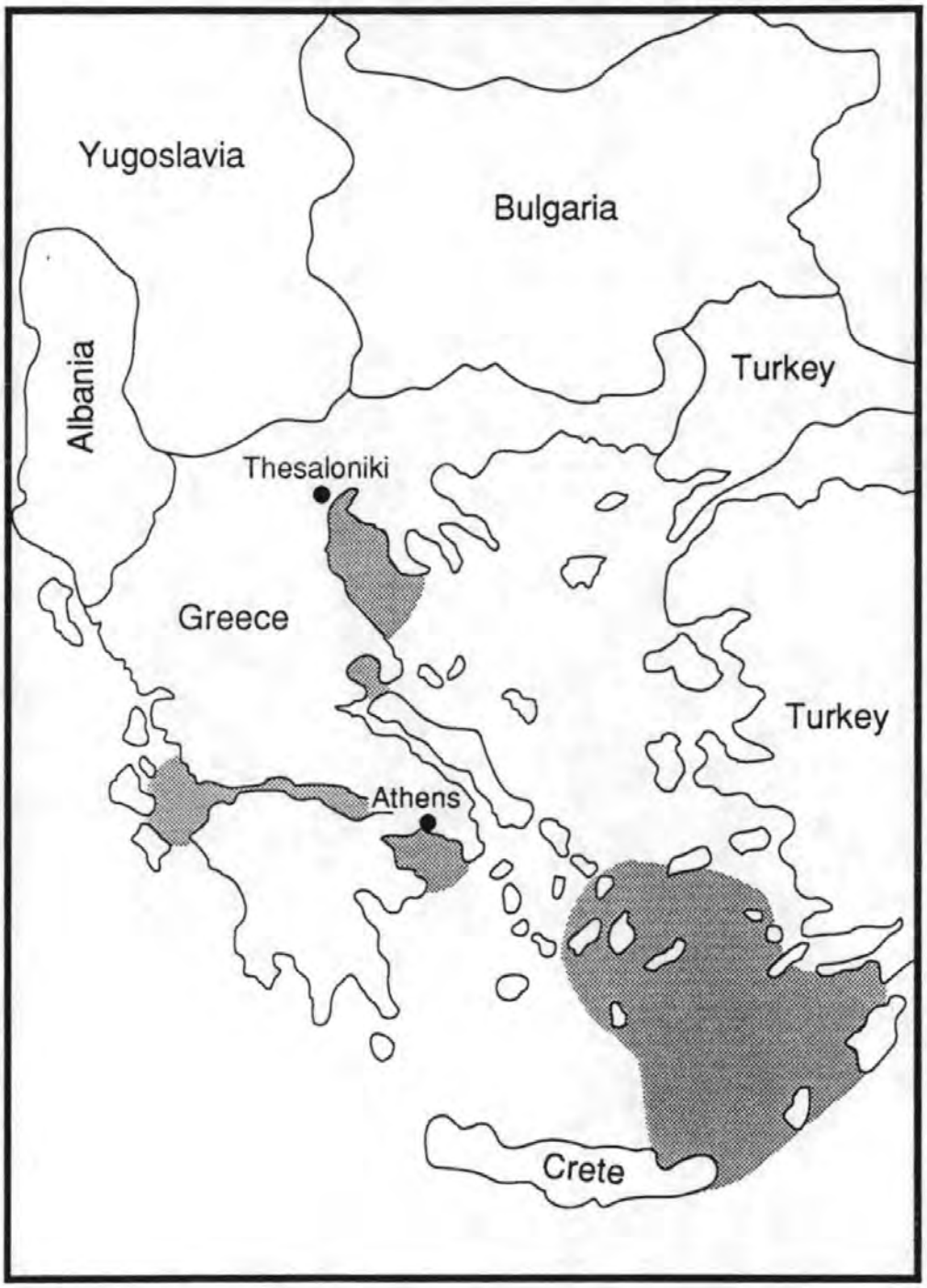

Figure 4. Distribution of Gilthead Seabream native to Greek Territorial waters

molars (rounded teeth) in the upper jaw and three to four rows in the lower (Figure 5). It is a carnivorous species feeding mainly on bivalve molluscs, crustaceans and gastropods; annelids and algae are only secondary food sources and the species may occasionally feed on small fish and insects (New, 1986).

Understanding the wild food sources of the gilthead seabream has made some contribution to our knowledge of its general nutrition and specific nutrient requirements. A number of workers have provided valuable data for related fish but there are only limited reports specifying the European gilthead seabream.

The following section reviews the literature applicable to qualitative and quantitative gross nutrient requirements and characteristics of this class of marine fish in general.

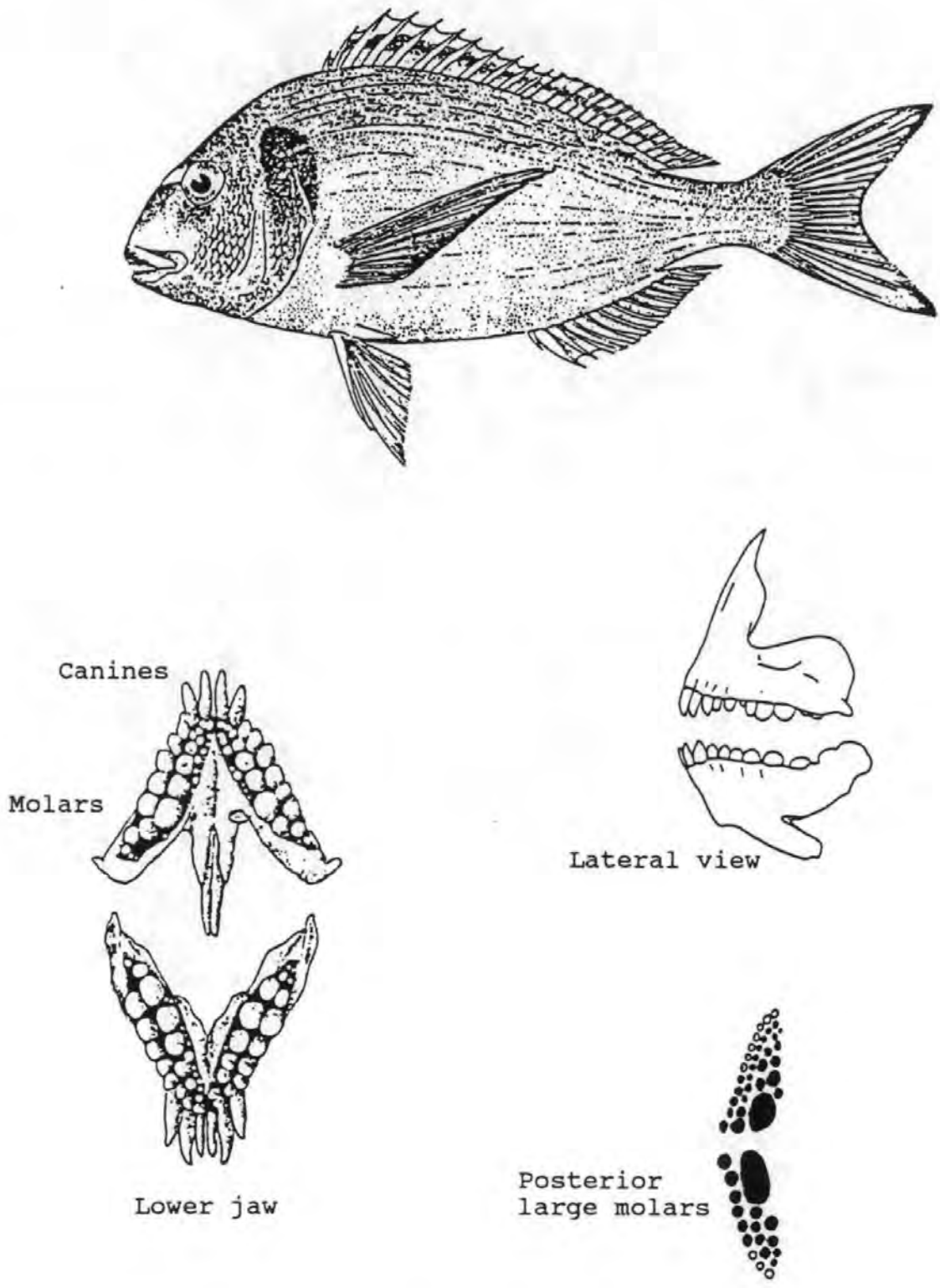

Figure 5. External body features and dentition of the Gilthead seabream Sparus aurata.

1.3. QUANTITATIVE AND QUALITATIVE NUTRITIONAL REQUIREMENTS OF SPARID TYPE FISH

1.3.1. Protein and essential amino acids

Dietary protein is always considered to be of primary importance in fish feeds as their requirements are higher than those of terrestrial farm animals (Wilson, 1985; Steffens, 1989). Protein is the basic building nutrient of any growing animal and muscle constitutes and anatomically the major component of the fish body. Dietary protein is utilized for the formation of new proteins, enzymes, hormones and reproductive organs, for the maintenance of depleted tissue, and as a major source of metabolizable energy. Consequently, protein usually accounts up to 68-85% of the dry matter component of a fish carcass (Jauncey and Ross, 1982).

Very little work has been directed into delineating the nutritional requirements of juvenile and growout stages of seabream.

Sabaut and Luquet (1973) experimented with juvenile seabream with an initial size of 26g and fed six semisynthetic diets containing 10 to 60% protein. Their investigation showed that the gilthead bream requires about 40% protein in a balanced diet. Other French workers have experimented with feeds containing 45 and 63% crude protein

(Koening,1973) while Kissil (1981) used protein levels of 48 and 54% in his formulations. These latter authors found that high protein diets were more effective for the growth of bream to a size of 5g and suggested reducing the dietary protein level beyond this size class of fish.

Kissil did not observe a specific demarcation at 5g, but the higher 54% protein level did provide better growth for fish attaining 15g. Although the qualitative requirements of marine fish for essential amino acids (EAA) have been shown to be the same as for other fish species, very little work has been published on their quantitative EAA requirements. Table 2 summarizes quantitative dietary EAA requirements of various species, including some work relating to the red sea bream (Pagrus major).

In continuation of the protein requirement research effort, Luquet and Sabaut (1973) investigated the qualitative amino acid requirements for the gilthead bream using radio labeled glucose (C14) as the carbon source. In this manner they determined that tryptophan, arginine, methionine and cysteine were essential in the diet of the gilthead. They further qualified the required levels for each of these amino acids by testing different levels in the diet in relation to growth response.

More extended work on red seabream (Pagrus major), a close relative to the gilthead, showed that with diets fed to

fingerlings, 35-70% protein (casein and gelatin) the growth rate of red seabream showed a linear elevation with the increase of dietary protein level up to approximately 55% protein (Yone, 1975). In a qualitative test for essential amino acids, it was found that these requirements for the red seabream are almost identical with those of salmonids, channel catfish and eel when expressed as a percentage of the dietary protein level (Sakamoto and Yone,1972). The recommended values for all the essential amino acids for red seabream are displayed in Table 2.

1.3.2. Protein - Energy Requirements

As mentioned previously, dietary protein is used by most fish as the primary energy source. Dietary lipid similarly provides a source of indispensible nutrients for maintenance and growth, but is also a major source of metabolizable energy (New, 1986). The dietary requirement of marine fish for carbohydrates which form the tertiary energy source, are somehow still uncertain and limited. Of the three components, protein is still the most expensive nutrient in terms of supplying the calorific needs of the fish.

Kissil and Gropp (1984) conducted two experiments in order to determine the optimal protein / energy ratios with complete feeds for the gilthead bream. With fish averaging 45g, superior growth was obtained by diets using 40% protein and 5% added fish oil. This latter diet represented a P/E

Table 2. Quantitative dietary essential amino acid requirements of various fish species (% of protein).

	Chinook Salmon	Rainbow Trout	Channel Catfish	Common carp	Red Bream	Gilthead Bream
Arginine	6.0	3.3	4.3	4.3	7.4[1]	5.0
Histidine	1.8	1.6[2]	1.5	2.1	3.4[1]	
Threonine	2.2	3.4[2]	2.0	3.9	3.4[1]	
Isoleucine	2.2	2.4[2]	2.6	2.5	4.6[1]	
Leucine	3.9	4.4[2]	3.5	3.3	6.8[1]	
Valine	3.2	3.1[2]	3.0	3.6	6.2[1]	
Lysine	5.0	4.2[2]	5.1	5.7	8.5[1]	5.0
Methionine	4.0	3.0	2.3	3.1	2.2[1]	4.0
Phenylalanine	5.1	3.1[2]	5.0	6.5	5.0[1]	
Tryptophan	0.5	0.5	0.5	0.8	1.2[1]	0.6

Data extracted from Wilson (1985).
[1] Data extracted from Yone (1975).
[2] Data extracted from New (1986).

ratio of 105 mg protein/ Kcal of gross energy. Using fish with a mean weight of 3 g the best results were obtained at higher protein level (44%) and 10% added fish oil or a P/E ratio of 95 mg protein/ Kcal energy.

Ina et al. (1981) used a feed for red seabream with a protein level of 51.7% and lipid level of 5.8%. The P/E ratio of the diet was 179 mg protein/ Kcal. The P/E ratios of several experimental diets tested for marine Percoidae are shown in Table 3.

In conclusion it would appear that the potential sparing capacity of lipid for dietary protein is not fully utilized in commercial feeds for marine species. This capacity, however, has been widely recognized for salmonid species where many workers have shown that significant reductions in protein levels may be achieved with the supplementation of dietary lipid in the form of marine oils (Reinitz et al., 1978; Gropp et al., 1982; Kellems and Sinhubber, 1982). Economically it would seem advantageous to lower protein and increase lipid levels simultaneously, bringing the P/E ratio within the range of 150-160mg protein/ Kcal (New, 1986).

1.3.3. Lipid and Essential fatty acids

It is well known that lipid is both important as an energy and essential fatty acid source for all farmed fish (Watanabe, 1989; Sargent et al., 1989). Until recently, corn

Table 3. Comparison of the lipid, protein and values and the P/E ratios of optimal diets for marine Percoidae (New, 1986).

Species	Animal size(g)	Crude lipid (%dw)	Crude protein (dw%)	P/E (mg protein/ Kcal)
Chrysophrys major	65	14	40	121
	Fingerling	6	60	198
	Adult	5	53	189
Sparus aurata	0.3-256	8	49	159
	2.6	8	40	135
	44	9	44	142
	Fry	11	55	164
	Fingerling	10	52	162
	100	9	44	146
Dicentrarchus labrax	5.5	12	40	135
	Fry	13	52	149
	75	12	53	159
	Larval	10	69	188
	5	10	62	177
	15	9	56	172
	30	9	53	166

oil and soybean oil were included in fish diets because these vegetable sources contain some of the essential fatty acids (EFA), of the n-3 and n-6 series mainly in the form of linolenic and linoleic acid. However, these oils are relatively low in the linolenic acid component and have a very low content of highly unsaturated fatty acids (HUFA) with chain lengths greater than 20 carbon atoms. These C20 and C22 extended polyunsaturates have proven advantages in the diets of marine fish fry and ongrowers (Izquierdo et al., 1989). Under natural conditions marine fish consume organisms rich in these polyunsaturated fatty acids and therefore the nutritive value of vegetable oil substitutes may be inferior to that of fish oils in respect to their EFA profile (Kalogeropoulos et al., 1990).

In order to consider the EFA requirements of sea bream we shall have to take into account the fact that marine species are reported to be less able to elongate and desaturate lower n-3 polyunsaturated fatty acids to C20 or C22 HUFA than freshwater fish (Millikin, 1982). An inability to convert C18 fatty acids efficiently, whether they be in the n-3, n-6 or n-9 series, has been shown for turbot by the latter author; similarly plaice failed to show increased levels of 20:5n-3 or 22:6n-3 in liver triglycerides when fed 18:3n-3 and 18:2n-6.

Only a few studies have been undertaken to determine the quantitative needs of Sparus aurata. It has been clearly

shown that long chain polyunsaturated fatty acids (n-3) are indispensable, but quantitative requirements still remain to be precisely defined. Kissil and Gropp (1984) concluded that 10% fish oil in the juvenile and 5% in the growout stages should be provided in practical diets for the gilthead sea bream, whereas Alliot and Pastoureaud (1984) concluded that optimal lipid levels for seabream lie in the region of 8-12% of the complete diet.

1.3.4. Dietary Carbohydrates

Since seabream are a carnivorous species, they are not generally accepted to be capable of efficiently utilizing carbohydrates as an energy source (Spannhof and Plantikow, 1982; Bergot and Breque, 1983). This inability to assimilate carbohydrates was considered to be primarily due to the difference in insulin secretion response (Furuichi and Yone, 1982) and in the relative activities of key hepatic glycolytic and glucogenic enzymes which are regulated by insulin (Furuichi, 1983).

In order to determine the ability of red seabream to assimilate carbohydrates, Furuichi and Yone (1971) fed fish with diets containing 10-40% glucose compared to a glucose free control group. Almost no difference was noted in the first 30 day period in the rate of growth and the efficiency of the feed, protein and calorific utilization. In the

following 20 day period, the 10% glucose diet group was inferior to the glucose free diet group. On the other hand fish fed diets containing 30 and 40% glucose, showed retarded growth and lower feed efficiency. An accumulation of a large amount of glycogen and lipid was found in the liver of bream fed high levels of glucose. It was also noted that the absorption rates of protein and glucose became lower with increased dietary glucose levels. Thus, the utilization of carbohydrates to spare protein is of limited value due either to their low digestibility or subsequent metabolic utilization.

Furuchi and Yone (1981) tested the blood sugar and plasma insulin levels of red sea bream fed different dextrin levels in feeds. Their results suggested that fish have a natural tendency to be diabetic and that long term feeding of high carbohydrate diets may reduce the net utilization of carbohydrates. Shimeno et al. (1977) found that apparent digestibility of carbohydrates and protein decreased together as starch levels increased in feeds for yellowtail (Seriola quinqueradiata). Mazzola and Rallo (1981), however, reported the successful use of a feed for rearing Sparus aurata with a total carbohydrate level of 31%.

Dietary fiber levels are usually kept low in fish feeds especially with those destined for carnivorous species, because this component appears to affect the digestibility of other nutrients (New, 1986).

The latter author suggests that it would be inadvisable to rise the dietary fibre much above 20 -25% because this may result in higher liver/body weight ratios and more fatty carcasses. Further research is needed on the complex relationships between protein, lipid and carbohydrates levels in complete diets.

1.3.5. Vitamins

Studies on fresh water fish, particularly salmonids, have served as the main reference for the qualitative and quantitative vitamin requirements of most fish (Halver, 1989). These values are often used by commercial feed manufacturers, but there is a paucity of information for the requirements of marine fish species. For pyridoxine, (vitamin B6), Kissil (1981) performed two experiments, testing the growth of gilthead seabream using purified test diets with varying levels of this vitamin. The two experiments showed that at vitamin levels below 8mg/kg dry diet, there was growth retardation, higher mortality, incidence of abnormal behaviour and visible histological change in peripheral nervous tissue. A similar series of experiments by the same authors for biotin, suggested a minimum dietary requirement between 0.37 and 0.21 mg biotin/kg dry diet.

Again, work on red seabream is more complete. Yone and Fuzii, (1974) studied the qualitative requirements and

deficiency symptoms of red seabream for water soluble vitamins. Red seabream showed a poor growth response and loss of appetite when the diet is deficient in B6, choline, pantothenic and, B12, inositol, nicotinic and B2, B1, or vitamin C ascorbic acid. On the other hand, with diets deficient in biotin, folic and or p-aminobenzoic and, the fish did not show any marked deficiency symptoms during a 102 day feeding trial.

Quantitative requirements for water soluble vitamins are still fragmentary. Fingerling requirements for inositol and vitamin B6 have been determined by Yone (1975) to be 55-90 and 0.2-0.5 mg/100g of diet respectively. Data on fat-soluble vitamin requirements are still incomplete for most marine species.

1.3.6. Minerals

Many nutritional studies have clarified the importance of inorganic elements on the growth of domestic animals and humans (Cho et al., 1985). However, relatively little attention has been paid to the requirements of farmed fish with respect to trace element requirements. This is probably because of the significant supply of minerals from the aquatic environment, which is particularly the case for marine fish where seawater is an abundant source of all trace elements. Since some elements, such as calcium, magnesium,

sodium etc abound in sea water, it can be expected that marine fish scarcely need these major elements in the diet. However, fish require a comparatively large amount of some minor elements in sea water, such as phosphorus and iron. An appropriate dietary phosphorus level range from a minimum of 680 mg% to a maximum of 1360 mg % has been identified for the red sea bream (Yone, 1975). A smaller amount of dietary phosphorus caused a decrease in hepatic glycogen and calcium, phosphorus and crude ash content in the vertebrae, as well as an increase in lipid in the liver, muscle and vertebrae (Sacamoto and Yone, 1978).

The iron content of sea water is very low. Thus an iron supplement in the diet is necessary to prevent microcytotic hypochromaemia and anicocytosis of erythrocytes, ie: characteristic features of iron deficient anaemia (Sacamoto and Yone, 1976). It was found that the iron requirement for Pagrus major is approximately 15mg/100g dry diet (Sakamoto and Yone, 1978). For other minerals, the sea water supply is sufficient and dietary supplements are not required (Sakamoto and Yone, 1979).

From the above sections it is clear that many areas need further investigation with respect to the nutrition of gilthead seabream. Of fundamental importance are questions concerning protein utilization, energy sources, essential fatty acid requirements, as well as the complete vitamin, mineral and trace element requirements.

Together, these nutrients form a balanced diet for fish and determine the gross profile of any test diet formulations for a specific type of fish.

1.4. FEED INGREDIENTS AND FEED FORMULATION FOR FISH

1.4.1. Feedstuffs for aquaculture

The feeds traditionally used for feeding monogastric farm animals are believed to be suitable for feeding most fish (ADCP, 1983). These materials include the feed grains, oilcakes and meals, animal byproducts (including fishmeal), and an assortment of industrial and agricultural waste products (Hardy, 1989; Kaushik, 1989; Lovell, 1989).

Typical examples of the most common feedstuffs with a potential use in aquaculture are presented below under appropriate categories :

Cereal grains and by products

Barley	(Hordeum vulgare)
Corn/Maize	(Zea mays)
Millet	(Pemisetum typhoideum)
Oats	(Avena sativa)
Rice	(Oryza sativa)
Rye	(Secale cereale)
Sorghum	(Sorghum bicolor)
Wheat	(Triticum aestirum)

The protein levels in cereal grains are low ranging from 8 to 12% with lysine and threonine generally being the first and second limiting essential amino acids (ADCP,1983). They

are rich sources of carbohydrate with levels of 60 to 80%. Cereal oils are normally unsaturated with linoleic and oleic acid being the predominant fatty acids present. They contain little calcium and are good sources of phosphorus and vitamins E and B (Allen, 1984). As with most plant feedstuffs they may contain a variety of endogenous antinutritional factors (Tacon, 1985).

Oil seeds and byproducts

Groundnut	(Arachis hypogaea)
Coconut	(Cocos nucifera)
Soyabean	(Glysine max)
Cotton	(Gossypium spp.)
Sunflower	(Helianthus annuus)
Linseed	(Linum usitatissimum)
Sesame	(Sesamum indicum)
Olives	(Olea europea)
Mustard	(Brassica spp.)
Rape	(Brassica napus)

Oilseeds differ from cereals in that lipid replaces carbohydrate as the major food reserve within the plant seed. They are rich sources of protein (20% to 50%) and relatively poor sources of carbohydrate. Lysine, methionine and threonine are usually the limiting amino acids (Bath, 1984). As with cereals, oilseeds are poor sources of calcium but

good sources of phosphorus, vitamin E and B. They also contain a variety of endogenous antinutritional factors (Tacon, 1985). The dried residue obtained by the removal of oil from these materials result in the respective meals and associated products.

Grain legumes

Pigeon pea	(*Cajanus cajan*)
Carob	(*Ceratonia siliqua*)
Chickpea	(*Cicer arietinum*)
Grass pea	(*Lathyrus sativus*)
Lentil	(*Lens esculenta*)
Lupin	(*Lupinus spp.*)
Pea	(*Pisum sativum*)
Ground bean	(*Kerstingiella geocarpa*)

Grain legumes are good sources of protein (20-26%), energy and several B vitamins. They are often considered as natural supplements to cereals, since, although they are usually deficient in the sulphur amino acids methionine and cysteine, they contain adequate amounts of lysine (Hardy, 1989).

Root crops

Elephant yam	(*Amorphophalus spp.*)
Sugar beet	(*Beta bulgaris*)
Taro	(*Colocasia esculenta*)
Yam	(*Dioscorea spp.*)

Sweet potato (Ipomoea batatas)

Potato (Solanum tuberosum)

Cassara (Manihot esculenta)

Root crops and tubers are poor sources of protein (2-10%), vitamins, calcium and phosphorus, but are rich dietary sources of potassium and digestible carbohydrates. Root crops also contain a variety of endogenous antinutritional factors (Tacon, 1987).

Vertebrate animal products

Meat meals

Meat and bone meals

Blood meals

Hydrolysed feather meals

Milk byproducts

Trash fish

Fish meals

With the exception of specific products such as blood meal and hydrolyzed feather meal, the majority of animal by-products have a well balanced essential amino acid profile and are good sources of protein, lipid, energy, minerals and vitamins (Harris, 1978; Tacon, 1987; Lovell, 1989).

Miscellaneous feedstuffs

Apart from the nutrients described in the preceding sections, there are also feed materials termed "non conventional" feedstuffs. These include leaf protein concentrates (LPC), fruits, some single cell proteins (SCP), algae and invertebrate food organisms (Cho et al., 1985; Tacon, 1987; Kaushik, 1989).

1.4.2. Diet Formulation

Diet formulation is a complicated process by which feed formulators select dietary ingredients and levels that may be combined to create a mixture that meets the nutritional requirements of the fish and is also palatable to ensure good feed intake. In addition, it must be pelletable, free from dust and easy to store and transport (Hardy, 1989).

Ingredients are chosen on the basis of nutritional profile ie: gross composition and nutritional value. Cost is also a major factor governing the choice of ingredients and materials in compounded feeds.

Efficient diet formulation requires the application of this knowledge for both the nutritional requirements of the fish in question and the nutrient content and availability of such ingredients (Kaushik, 1989).

The availability of nutrients in a given feed ingredient is an important aspect that must be considered for effective

feed formulation. Such factors as processing technology and interaction with other dietary ingredients can reduce the availability of a specified nutrient. However, determining dietary availability under aquatic conditions involves feeding experimental diets to fish and measuring such parameters as digestibility, enzyme activity, or tissue nutrient saturation (Hardy, 1989). This is a very complex objective and has difficulties reviewed in later sections.

Ingredients and finished diets can also be evaluated by a variety of chemical and biological tests.

1.5. EVALUATION OF MAJOR FEED INGREDIENT COMPONENTS OF FISH DIETS

1.5.1. Gross chemical methods

Chemical methods of evaluation are those performed in vitro and are used mainly for quality control and to predict nutritional value of a feed mixture or individual feed ingredient.

More commonly, proximate analysis is a partitioning of the major nutrient components of a feed into moisture, crude protein, ether extract, crude fiber, ash and nitrogen free extracts (NFE).

The procedures used throughout this work are described in AOAC (1984) and are summarized below.

Moisture is defined as the loss in weight of a sample after oven drying for 24 hours at 105^{o} C to a constant weight.

Crude protein is estimated by the Kjeldahl procedure in which the nitrogen (N) content is directly measured and converted to protein content using the conversion factor 6.25.

Ether extract refers to the fat or lipid content of a sample and is extracted on a soxhlet apparatus using hot petroleum ether as a solvent. After extraction, the ether is evaporated and the weight of the extracted material determined.

Crude fibre is the residue obtained after boiling the sample in weak acid followed by boiling in weak base. The remaining material minus the inorganic residue represents the value for crude fibre in the sample.

Ash represents the inorganic component of a sample material and is the residue obtained by its ignition at 550^{o} C. It contains essential and non essential elements and also any toxic elements.

NFE includes the simple sugars, compound sugars and soluble polysaccharides, such as starch. NFE in most cases is determined indirectly by subtracting the sum of all other categories from 100% of the sample composition.

It should be noted that more refined analysis frequently involves the chemical estimation of individual essential nutrients such as amino acids, fatty acids, minerals and vitamins. However, chemical methods estimate the levels of essential nutrients in a mixture or a single ingredient but these are not always equal to the levels that are available to fish (Hardy, 1989; Steffens , 1989).

1.5.2. Qualitative tests

1. Protein.
   a. Pepsin digestibility estimates the degree of protein availability, and is measured <u>in vitro,</u> by digesting a

sample in a warm solution of pepsin.

b. Determination of antinutritional factors in feeds such as trypsin inhibitors and urease.

c." Available" lysine methods measure the amount of lysine that can be digested by the fish after heat processing. By comparing the total and "available" lysine levels, the quality of the protein is judged.

d. The cresol red value is used to judge the amount of heat used to process protein concentrate feedstuffs.

2. Lipids.

The two major concerns are hydrolytic and oxidative rancidity. Several methods have been developed to detect lipid rancidity such as peroxide values, thiobarbituric acid test (TBA), anisidine value, Kries test as well as many others.

3. Antinutritional factors and toxins.

A number of analytical methods have been developed to determine the presence of antinutritional factors and toxins in a feed sample. These include assays for glycosides such as, gossypol, glucosinolates and phytic acid, tannins, saponins etc.

1.5.3. Biological evaluation

The biological evaluation of feed ingredients and finished diets typically involves feeding fish in experimental trials and analyzing some aspect of fish performance and diet digestibility. Biological evaluation methods can be divided into 3 general categories according to many researchers; eg:- (Gropp, 1979; Gropp and Tiews, 1981; Jauncey, 1982; and Hardy, 1989) :

1. Retention studies, in which the deposition of a nutrient in the carcass over time is measured.
2. Deficit studies, in which the various losses of ingested food via the faeces, urine and gills are measured, and
3. Performance studies, in which some measure of growth is used to evaluate and compare feeds (Hardy, 1989).

The biological evaluation methods are organized into 3 groups: general methods used for various nutrients, methods used specifically for proteins, and methods used for energy budget studies.

1.5.3.1. General parameters and techniques

a. Growth over a specific time period is measured directly as the live mass gain of fish fed the test diets.
b. Specific growth rate (SGR) is used to compare growth on a relative daily basis, using the expression as percent

increase in live weight gain over a fixed time period. ie:-

$$SGR = \frac{\ln Wf - \ln Win}{T} * 100$$

where,

Wf : final weight

Win : initial weight

T : duration of trial (days)

c. Feed efficiency (FE) relates the ability of feeds to support weight gains and is defined as the ratio of the live weight gain of the fish to the dry feed intake.

d. Digestibility coefficients (DC) are used for both feed ingredients and complete feeds. An inert material, usually chromic oxide Cr_2O_3 is added to the feed for such tests. Faeces are collected and the marker concentration of both the feed and faeces are determined. The digestibility coefficient is then determined for a designated nutrient by relative concentration ratios using the formula,

$$DC = 1 - \frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in feces}}$$ for dry matter and

$$DC = 100 - \left[\frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in faeces}} * \frac{\%\text{nutrient in faeces}}{\%\text{nutrient in feed}} * 100 \right]$$

for a designated nutrient.

The value obtained by the above procedure is termed the "apparent" digestibility coefficient, because no correction has been made for endogenous fecal excretion of nutrients.

e. Carcass deposition of specific nutrients in the carcass of fish over a specific time period evaluates the net availability of each component, usually protein and energy. Carcass deposition can also be expressed as apparent retention (AR)

$$AR = \frac{C(end) - C(start)}{N(intake)} \quad \%$$

where :

C(end) : carcass nutrient content at end of experiment

C(start) : carcass nutrient content at start of experiment

N(intake): nutrient intake during the experimental period.

1.5.3.2. Methods used for protein utilization.

a. Biological value (BV) measurements are used to determine the % of absorbed nitrogen (N) retained by a fish by measuring nitrogen excreted during a test period. BV is calculated as

$$BV = \frac{\text{food N}-(\text{fecal N}+\text{urinary N}+\text{branchial N})}{\text{food N}} * 100$$

b. Protein efficiency ratio (PER) is a measure of the weight gain per unit protein fed and is a useful method to compare protein sources in a trial.

$$PER = \frac{\text{Weight gain (g)}}{\text{Protein fed}}$$

c. Net protein utilization (NPU) is the protein gain during an experimental period per unit protein ingested by the fish. This is a more specific index for protein efficiency compared to PER. The latter accounts for live weight changes relative to protein intake which may be affected by body composition.

$$\text{Apparent NPU} = \frac{P(end) - P(start)}{P(fed)} * 100$$

where:

P(end) : Protein content (g) of fish at end

P(start) : Protein content (g) of fish at start

P(fed) : Protein fed

1.5.3.3. Methods used for energy utilization.

The question of how much energy is available to fish from ingredients and finished feeds is of critical importance in feed formulation. Dietary energy is expressed as Gross energy (GE), Digestible energy (DE), Metabolizable energy (ME), and net energy.

For ingredient and diet evaluation, determining net energy using comparative slaughter techniques, or estimating digestible energy and metabolizable energy for production, are methods that are suitable for use in most laboratories (Hardy, 1980).

1.5.4. Economic evaluation

Ideally feed formulation and complete diet evaluation should be based on a least cost per unit of product basis. At the present time, insufficient data exists to make this approach feasible. It is important to thoroughly investigate this area so that more realistic economic evaluation of fish feeds will be possible in terms of effective diet formulations. This is already an established practice in the animal and poultry feed industry and is also becoming

increasingly applicable to farmed fish.

The previously defined parameters serve as the main basis for most nutritional investigations including fish and form the standard methods employed in the following chapters.

Given the stated importance of the gilthead sea bream as a principle species within the mariculture operations of southern Europe, a series of investigations were conducted to assess the feeding value of selected animal and plant materials and byproducts in experimental diets for this species. This would lead to the objective of practical feed formulations for sea bream based on these findings.

CHAPTER 2

2. EXPERIMENT 1

AN INVESTIGATION TO DETERMINE THE DIGESTIBILITY COEFFICIENTS OF VARIOUS RAW MATERIALS FOR THE GILTHEAD SEA BREAM (Sparus aurata)

2.1. ABSTRACT

A number of raw materials were tested to obtain the apparent digestibility coefficients of protein, lipid, carbohydrate and gross energy (GE) for sea bream (average weight of 45g) under controlled conditions. The experimental diets consisted of a fixed ratio of a control basal diet and each of the raw materials under test. Digestibility of a nutrient contained in complete mixtures was first determined and then the digestibility of the nutrient content in the raw material was found by calculation from its contribution to the amount of nutrient in the mix. In the case of protein digestibility assessment, a standard in vitro method was also included for comparison.

The protein digestibility coefficients were generally high, with certain exceptions such as the low digestibility of feathermeal and tomato pulp found for sea bream, which could

be further examined.

Carbohydrate digestibility ranged from 40-70% for the test diet mixes. The reproducibility of the analytical method used was not however sufficient to allow more detailed comparisons to be made for specific raw materials. Lipid digestibility was generally, as expected, high for most of the tested ingredients. A reliable chemical oxidation method was found to be an appropriate procedure for gross energy digestibility coefficient determinations compared to standard bomb calorimetric methods. Values ranged from 17% to 80% for the feed mixtures and from 6% to 80% for the individual raw materials.

2.2. INTRODUCTION

Very few nutrients are utilized by an animal in the form in which they are ingested (Smith, 1979). Digestion processes must degrade protein molecules into free amino acids, complex carbohydrates must be broken down to simple sugars and fats must by hydrolyzed to fatty acids, mono-glycerides and di-glycerides before absorption and assimilation. The nutritional value of a feed, therefore, is dependent not only upon the nutrient content, but also upon the ability of an animal to digest and absorb the nutrients from the feed. (Smith, 1978; Kirchgessner et al., 1986).

There is considerable variation in the ability of different species to digest protein because of the nature and activity of their protein hydrolyzing enzymes in the gastrointestinal tract (Smith, 1989).

The gastric phase of proteolysis is initiated by the combined action of pepsins initially secreted in zymogen form and gastric acid (HCl) (Ash, 1985). The extent of proteolysis within the stomach is related to a number of factors such as the combined secretory rate of acid and pepsin, retention time, feed intake etc (Smith, 1967). The primary role of the gastric phase is in the preparation of susceptible peptide linkages for subsequent hydrolysis within the small intestine. However, Austreng (1978) and Dabrowski and Dabrowska (1981) suggested that to some extent, protein

digestion and even amino acid absorption occurs within the stomach of rainbow trout (Oncorhynchus mykiss). At the intestinal phase of protein digestion the endo and ecto-peptidases of the pyloric caecae provide a rich source of pancreatic enzymes (Overnell, 1973).

The spectrum of enzymes contributing to the luminal hydrolysis of dietary proteins in fish include : trypsins, chymotrypsins, carboxypetidades, and elastases (Yoshinaka et al., 1982; McLeese and Stevens, 1982) which are considered to be of pancreatic origin. Much less information is available on enterocyte associated enzymes for fish.

Very limited work has been carried out on the amino acid digestion and absorption within the gastrointestinal tract of fish. Certain amino acids may not be available to the fish because of incomplete protein digestion, or possibly some non protein compounds may bind certain amino acids into a non utilizable form (Sawer and Thacker, 1986; Wilson, 1989). For example, when some proteins are overheated in the presence of reducing sugars, the reducing sugars may react with the ϵ-amino group of the essential amino acid lysine, thus reducing availability.

A considerable amount of attention has been focused on the question of protein digestibility in fish and such work demonstrates that, with few exceptions, most dietary proteins exhibit high true and apparent digestibility coefficients (Ash, 1985). Table 4 summarizes protein digestibility

Table 4. Apparent Protein digestibility coefficients (DCp) of important ingredients for carnivorous fish (Kaushik, 1989).

Raw Materials	Crude protein (%)	DCp (%)
Fishmeals	70-75	80-90
Fish solubles	38	70
Blood meal (drum dried)	92	32
Blood meal (spray dried)	95	86
Meat and bone meals	48-53	69-75
Poultry byproducts	43-50	65-75
Corn gluten	69	87
Distillery solubles	25	70
Rapeseed meal	38	76
Dehulled soybean meal	46-55	75-85
Wheat middlings	16-19	65-68
Brewer's yeast	48-51	83

coefficients of some important ingredients for carnivorous fish.

Compared with terrestrial animals fish poorly utilize dietary carbohydrates. Although most fats and proteins can be digested by up to 90-98%, digestibility coefficients for carbohydrates are often much lower (Spannhof and Plantikow, 1983). Although omnivorous fish show some degree of adaptation of their digestive system in response to changes in dietary carbohydrate content, it is unclear whether carnivorous fish are capable of such adaptive flexibility (Buddington and Hilton, 1987). Gastro-intestinal regions noticable for carbohydrate digestive enzymes in fish are the pyloric caecae, pancreas and intestinal mucosa. Arthur and Philips (1969) stated that the digestive enzymes presented in the digestive tract of trout (Oncorhynchus mykiss) were sucrase, lactase and amylase. They also stated that not all the above enzymes were present at similar levels, and there was considerably more maltase activity than lactase. Shimeno et al. (1977) found that yellowtail (Ocyurus chrysurus) possessed high activities of gluconeogenic enzymes and pepsin, and low activities of the glycolytic enzymes, pentose cycle dehydogenases and amylase. In red seabream (Pagrus major), Kawai and Ikeda (1971) reported high activities of maltase in the pyloric caecae and intestine and less activity in stomach. Amylase activity was also depleted in hepatopancreas. Finally the activity of amylase which

hydrolyses starch was also reported to be higher in omnivorous fish such as carp (Cyprinus carpio) than in carnivorous fish such as trout, eel (Anguilla anguilla) and yellowtail (Kitamikado and Tachino, 1960; Negayama and Saito, 1968; Shimeno et al., 1977). Further information on starch digestibility and utilization would be useful in order to formulate cost effective diets. Factors such as carbohydrate source, physical state and dietary level can affect the digestibility.

Processing technology can improve starch digestibility for carnivorous fish (Bergot and Breqie, 1983). Any technological treatment that might decrease the complexity of the dietary starch can considerably improve its digestibility (Kaushik et al., 1989). Digestibility of starch in diets is greatly increased by heating or cooking procedures to achieve gelatinization (Chiou and Ogino, 1975; Jollivet et al., 1988).

Fats as a group, have less variation in chemical composition than proteins or carbohydrates. Therefore the digestion and absorption of fats is probably more uniform compared to that of both carbohydrate or protein (Smith 1979). Fish oils contain large amounts of mono-and polyunsaturated fatty acids and are particularly well absorbed in fish, the apparent digestibility coefficients (DC) being 85 to 96% (Cho and Slinger, 1979).

Lipase activity is present in extracts from pyloric

caecae, pancreas and upper intestine of various species of marine fish. Histochemical studies in teleosts have shown lipase activity in these tissues and in stomach and hepatopancreas tissues (Sargent et al., 1989). Areas of digestive tract in tilapia with high lipolytic activity also have a general esterase activity too (Hirji and Courtney, 1983). High lipase activity occurs in the pyloric caecae of many fish species establishing a major role for this tissue in lipid digestion (Mankura et al., 1984). All fish livers produce bile which is stored in the gallbladder. Fish bile is alkaline, containing enzymes and the added function of emulsifying lipids (Sargent et al., 1989). The hydrolysis of wax esters has been demonstrated in intestinal fluid from anchovy (Engraulis encrasicalus), striped bass (Morone saxatilis) and pink salmon (Oncorhynchus gorbusha) (Patton et al., 1975), as well as carp hepatopancreas (Kayama et al., 1979), pyloric caecae extracts from rainbow trout (Tocker and Sargent, 1984) and in various intestinal extracts from several other fish (Mankura et al., 1984). Phospholipids can be a major source of essential fatty acids for fish and are vital for cell membrane biosynthesis. However, although phospholipase A1 and A2 activities have been found in trout tissues, the intestinal extracellular phospholipase activity in fish has not been studied extensively (Neas and Hazel, 1984). Finally, sterol ester hydrolase activity has been found in the pyloric caecae extracts from rainbow trout

(Tocker and Sargent, 1984).

The fundamental digestion of major nutrients in fish is known to be affected by biotic as well as abiotic factors. This has been reviewed by Fange and Grove (1979) and by Windell et al. (1978). It has been shown that meal size, fish body weight and composition of the diet are all important variables that must be considered. External factors such as temperature and salinity may also influence the rate of digestion in fish (Jollivet et al., 1988)

Methods of digestibility measurement in fish follow one of two basic approaches: Either by direct or indirect measurement (De la Noue and Choubert, 1986). The direct method consists of quantitative collection of faeces. The most common method to measure digestibility of feedstuffs is the indirect method, i.e. to include within the diet a substance which is neither digested nor absorbed and then follow its increasing concentration through the digestive tract as digestible components of the diet are selectively removed. Chromic oxide (Cr_2O_3) has been the most widely used exogenous marker although there has been a recent interest in the use of other markers such as crude fibre, polyethylene and acid insoluble ash (Tacon and Rodriges, 1984; Smith, 1989). This has led to much debate concerning the validity of reported results.

It is essential that the collected faeces should represent

quantitatively the undigested residue of the food consumed. This is in practice difficult to achieve because of the problems associated with the aquatic environment. Faeces voided naturally into the aquarium water can be collected by fine dip net, syphoning, filtration columns, settling columns and mechanically rotating filter screens (Kaushik and Luquet, 1976; Cho and Slinger, 1979). The main problem with collection of naturally voided faeces is leaching of nutrients leading to an overestimation of digestibility. This problem of leaching has led several investigators to develop methods for collecting faeces from the intestine before it is naturally expelled from the fish. Faeces can be collected by killing fish and dissecting the gut contents or by manual stripping from live fish where faeces are expelled by applying pressure to the abdominal cavity between the pelvic fins and the vent (Smith, 1979). Unfortunately manual stripping may lead to the collection of incompletely digested food and samples contaminated with body fluids resulting in an underestimation of digestibility. However this method is particularly suited to salmonids due to their anatomical features.

Windell et al. (1978) described an anal suction method for removing the lower formed faecal pellet from rainbow trout. A specialized method was developed by Smith et al. (1980). This depends upon an elaborate metabolic chamber for fish which permits separate and quantitative collection of

faeces, urine and gill excretions. However this system poses a considerable problem in that only single fish can be used, in sealed units where conditions might be particularly stressful for achieving reliable data.

Until recently all materials, including fishmeal and alternative protein sources, received little or no treatment resulting in variable digestibility coefficients and subsequent performance. Nowadays, almost all the materials are processed and can be separated into two broad categories. The first involves the use of conventional feed ingredients, but maximizing their nutritional value by processing technology. The second involves the use of a new generation of unconventional feed ingredients obtained from biotechnological advances in the pharmaceutical and petroleum refining industries and also byproducts resulting from various brewing and distillation industries (Lovell, 1980).

The first stage of processing usually involves grinding which facilitates the destruction of heat labile anti-nutritional factors such as trypsin inhibitors and haemagglutinins in plant materials. These are invariably present and grinding also improves nutrient digestibility by increasing the surface area of the particles (Tacon and

Jackson, 1985). This improves feed acceptability and palatability. Following grinding there are basically three different heat treatment processes applicable, depending on the material in question; these are micronisation, extrusion and expansion and are mainly applicable to plant oil seed products such as soyabean meals.

It was the aim of this current investigation to determine the digestibility coefficients of the major nutrient components ie: protein, fat, carbohydrate and the energy content within selected animal and plant raw materials for possible use with the gilthead seabream (Sparus aurata). All these materials are currently employed in the animal feed industry and have further potential for inclusion in practical fish diets. Many of these products are readily available to the Greek market and used by the compounding trade.

2.3. MATERIALS AND METHODS

2.3.1. Experimental Fish

Gilthead seabream (Sparus aurata), of mixed sex obtained from the Selonda fishfarm in Greece were held in the experimental facilities of the National Center for Marine Research (NCMR) in Athens. The fish averaging 45g were randomly distributed into four 75 l digestibility tanks. Each tank accommodated 22 fish. Prior to the study the fish were kept on a standard commercial marine fish diet (Aqualim SA. France).

2.3.2. Holding facilities

The experimental system (Plate 3) consisted of four cylindroconical tanks with capacity 75 l and a 400l reservoir tank. The water recirculated with a flow of 2.5l/hr and a complete water exchange was applied at the end of each feeding day. The photoperiod was set to 8hrL:16hrD and the temperature controlled at 20^{o} C throughout the experimental period. At the end of each day the tanks were thoroughly cleaned in order to eliminate possible contamination. Faeces were collected the next morning from the settling silicone tubing at the bottom of the conical part of the tank. Fish were adapted for 4 days to each diet and ration, and the faeces collected for periods of 8-15 days. The diets were fed

Plate 3. Collection of faecal material in digestibility experiment.

three times daily , totalling 2% of the body weight per day. Every 14 days the fish were aneasthetized and individually weighed. The quantity of the feed was then adjusted to meet the fixed feeding level adopted for the study.

2.3.3. Treatment of faecal material

The faecal samples were stored frozen at -18 C and then lyophilised. After grinding the samples were analyzed in duplicate for each daily collection for protein and were pooled together and analyzed in triplicate for fat, carbohydrates, energy together with chromic oxide (Cr_2O_3).

2.3.4. Experimental diets

The chemical analysis of experimental diets containing the raw materials under test and of the raw material are summarized in Tables 5 and 6. A mixture of 40% fishmeal and 60% wheat meal was employed as the fixed component of the basal test diets at an inclusion level of 50%, the remaining 50% was contributed by each of the different test ingredients (Table 7). This 40% fishmeal/ 60% wheat meal was also used as the complete basal control diet. One per cent of chromic oxide (Cr_2O_3) was incorporated as the internal marker for these studies as well as sufficient amounts of vitamins and minerals (Table 15).

The test materials were respectively feather meal, three

Table 5: Composition of raw materials used in experimental digestibility studies.

ANIMAL BY-PRODUCTS

BY-PRODUCT	Dry weight	Protein	Lipid	Ash	Fibre	NFE
Poultry byproduct meal	94.86	51.65	29.50	10.50	1.20	-
Feather meal	91.20	82.13	1.80	4.65	2.60	-
Herring meal	94.04	68.16	12.44	13.01	1.00	-
Meat and Bone meal (C)	97.54	59.35	8.90	24.84	5.13	-
Meat and Bone meal (E)	95.00	59.00	10.00	32.00	2.00	-
Meat and Bone meal (L)	93.00	46.00	12.50	33.00	1.00	-
Poultry meat meal (PMM)	95.00	65.27	14.22	15.79	2.00	-
Lipromel	90.00	55.24	30.00	8.00	2.00	-
Denatured milk	94.80	31.83	0.39	9.75	10.37	42.46

PLANT BY-PRODUCTS

BY-PRODUCT	Dry weight	Protein	Lipid	Ash	Fibre	NFE
Sunflower meal	90.77	32.10	1.40	7.24	21.00	29.00
Cottonseed meal	87.18	43.74	1.16	6.09	4.90	31.30
Soybean meal	87.18	43.74	1.16	6.09	4.90	31.30
Tomato pulp meal	94.33	21.40	8.44	7.97	37.30	19.20
Wheat middlings	89.83	16.59	4.12	5.00	6.90	57.20
Corn gluten meal	93.81	65.73	4.56	5.32	3.81	14.39
Flaked maize	90.45	8.08	3.62	1.09	16.77	60.44
Full fat soya meal	92.59	34.42	23.11	5.29	4.56	22.92
Maize gluten extract	90.59	19.03	7.43	8.35	24.46	30.73

N.F.E. Nitrogen free extracts (determined by difference).

Table 6. Proximate analysis of test feeds (Diets) used for the adult Seabream digestibility trial.

	Moisture	Protein	Lipid	Ash	NFE
Control 1	9.89	38.20	5.75	7.28	38.90
Feather meal	11.01	54.06	3.18	5.97	25.78
Meat and bone (C)	13.12	50.91	2.41	18.39	15.17
Meat and bone (L)	4.99	45.82	7.34	20.27	21.58
Meat and bone (E)	4.89	43.93	6.19	19.14	25.28
Lipromel	9.28	49.91	17.15	7.19	83.53
Pultry meat meal	8.73	52.25	8.02	10.52	20.48
Poultry byproduct meal	12.17	43.89	17.36	8.89	17.69
Control 2	14.14	36.15	6.02	7.62	36.07
Cotton seed meal	12.61	33.60	2.87	6.69	44.23
Sunflower seed meal	12.70	34.56	3.24	7.26	42.24
Corn gluten meal	13.78	49.62	5.15	6.30	25.15
Cotrol 3	16.69	39.78	5.42	7.03	31.08
Tomato seed meal	11.74	26.76	7.09	7.86	46.55
Soya bean meal (Solv. extr.)	15.84	38.72	3.45	6.69	35.30
Denatured milk	21.84	29.81	3.07	8.52	36.76
Control 4	12.94	35.45	5.26	7.83	38.52
Flaked maize	13.09	20.71	3.41	4.65	58.14
Full fat soya meal	13.04	33.86	14.43	6.29	32.38
Maize gluten extract	15.28	25.56	6.59	7.82	44.75

Nitrogen Free Extracts (NFE) calculated by difference

Table 7. Composition of control diet mixture.

Ingredient	Inclusion(%)
Wheat meal	58.74
Fish meal (Brown Herring)	39.16
Vitamin premix	0.20
Mineral premix	0.30
Choline chloride	0.50
Chromic oxide	1.00
Ascorbic acid	0.10

types of meat and bone meals (MBL, MBC and MBE; the specifications of these materials are given in page 99), a commercial animal meat/fat blend, a poultry meat meal as well as a poultry byproduct meal, denatured milk and fishmeals representing the animal protein range, and cottonseed meal, sunflower meal, corn gluten meal, tomato pulp, solvent extracted soyabean meal, flaked maize and full fat soya meal as the plant protein selection.

All the dietary ingredients were mixed thoroughly and an appropriate amount of water was included, to form a soft dough. A Hobart bench food mixer (model no. A120) was used to blend and cold pellet each diet. The resulting moist pellets were dried in a convection dryer at 40^{o} C to achieve the desired moisture content. The pellets were then stored for the subsequent trials at -20^{o} C.

2.3.5. Analytical methods

Standard AOAC methods (1984) were used to obtain the proximate analysis of the diets and raw materials. Crude protein content (N*6,25) of the diets, ingredients, and faecal samples were determined according to the standard AOAC Kjeldahl method for Nitrogen (N). Crude fat content was determined using a soxhlet apparatus with petroleum ether extraction for the diets and the raw materials. For the faecal material, however, due to the limitations of the quantities of the samples, a micromethod for total lipids was

developed (Boehringer kit, cat no 124303). The method was based on the principle that lipids react with sulfuric acid, phosphoric acids and vanillin to form a pink colored complex and is described below :

Lipid (colourimetric assay)

100 mg of the sample was put into a centrifuge tube and 3 ml of chloroform / methanol (2/1) was added. The sample was then macerated for five minutes over ice. After 10 minutes of centrifugation at 1500 rpm, the supernatant was transferred into a preweighed tube and the volume of the solvent estimated. 50 ml of the standard total lipid solution was also transferred into a tube. 2ml of H_2SO_4 was added into each tube using an automatic pipette and well mixed. The samples were then stoppered and left into a boiling water bath for 10 min. After cooling 10ml of the samples and standards were again transferred into a new set of tubes and 2.5 ml of vanillin was added, mixed and left for 30 minutes. Absorption was measured in a spectrophotometer at 530 nm and the percentage concentration of lipids calculated by the following formula :

$$\text{Lipids (\%)} = \frac{\text{As} * 0.5 * \text{V} * 100}{\text{Ast} * 0.3 * \text{W}}$$

where,

As : Absorbance (Sample)

Ast : Absorbance (Standard)

V : Volume of the solvent (ml)

W : Sample weight (mg)

Available carbohydrate

Carbohydrates were determined for the diets, raw materials and faeces using a colourimetric procedure modified by Hudson et al. (1976). The method relies on the enzymatic degradation of starch into free monosaccharides which can then be assayed colourimetrically. Free glucose is also measured in this procedure.

Replicate portions of 50 mg of finely ground sample were weighed into screw cap vials and 5 ml of buffer (0.2 M sodium acetate, adjusted to pH 5.5 with glacial acetic acid) were added. The vials were then stoppered and left for two hours in a sand bath at 150^{o} C under continuous stirring. Whilst the samples were still hot, they were macerated for 3 minutes and more buffer was added to bring the final volume to 10 ml. The samples were then diluted ten fold. A 0.5 ml aliquot of the diluted samples were taken for glucose determination. 0.5 ml of 5% phenol reagent was added, mixed thoroughly and followed by 2.5 ml of concentrated H_2SO_4. The samples were mixed again, left to cool and the colour intensity measured using a spectrophotometer at 485 nm. Reagent and pigment blanks and the glucose standards were all treated using the

same procedure as above. The percentage concentration of glucose was calculated according to the following term:

$$\text{Glucose (\%)} = \frac{As * Gs * D}{Ast * W * 10}$$

where,

As : Absorbance (Sample)

Gs : Glucose concentration of standard (g/ml)

D : Dilution factor

Ast : Absorbance (Standard)

Gross energy

Gross energy was determined by adiabatic bomb calorimetry for the diets and raw materials and by a wet oxidation method (O'Shea and Marguire, 1962) modified by the author. Faeces were also analyzed using the wet oxidation method. The method was especially adjusted for micro sample quantification such as faecal material analysis and is described below :

Wet oxidation procedure

50 mg of sample were placed in a 250 ml conical pyrex flask, previously calibrated to 100 ml. 8 ml of potassium dichromate (1.5 N), was transferred into the flasks, followed by 16 ml of concentrated H_2SO_4. The mixture was

heated in a waterbath at 60^{o} C for 10 minutes and left to cool. The solution in each flask was then made up to 100 ml with distilled water and left to cool again. 20 ml of 20% potassium iodide solution was then added and the flasks were placed in a light proof cabinet for 25 minutes. Finally, 50 ml of distilled water was transferred to each flask and the liberated iodine titrated against 0.3 N sodium thiosulphate. Potassium dichromate is reduced in quantitative amounts as it is used to oxidize food molecules. Unreduced dichromate reacts with iodide, converting this to iodine, which is then estimated by titration with thiosulphate. Thus to calculate the quantity of 1.5 N dichromate used to oxidize a known weight of material, the sample titre is subtracted from the blank titre and the resulting figure divided by 5 to adjust for normality. This figure is then converted to energy units by applying an oxidation coefficient "C" which was, initially, obtained from O'Shea and Maguire (1962). This coefficient relates the quantity of dichromate needed for oxidation to its energy content as determined by direct calorimetry. Thus, C is measured in units of ml $K_2Cr_2O_7$/KJ. If x is the amount of dichromate used to oxidize an unknown sample, then x/C is the energy value. Oxidation coefficients are derived from correction equations to compensate for the incomplete oxidation of protein by dichromate. In the present study a correction coefficient was derived, which differs from the one that used by O'Shea and Maguire

(1962). The need for a different coefficient was introduced due to slight changes of the chemical procedure.
Fifteen samples were measured for their energy content by bomb calorimetry and the corresponding correction coefficient was applied using the previous methods and following expression:-

$$C = \frac{\text{ml } K_2Cr_2O_7}{\text{energy content (KJ)}}$$

Table 8 shows the results for a series of controlled protein levels based on purified ingredients and some selected raw materials for comparison.

Table 8. Crude protein and "C" values for a series of purified ingredients and some selected meals.

Sample	"C"	CP (%)
100% casein	3.78	86.5
70% casein, 30% cellulose	4.29	60.0
55% casein, 45% cellulose	4.38	47.4
40% casein, 60% cellulose	4.80	25.6
30% casein, 70% cellulose	4.79	23.5
15% casein, 85% cellulose	5.12	13.2
5% casein, 95% cellulose	5.36	4.2
2% casein, 98% cellulose	5.73	1.7
100% cellulose	5.59	-
Soyabean meal (solv. extracted)	4.49	51.1
Meat and bone meal (C)	3.90	63.2
Sunflower meal	4.41	36.9
Fishmeal (white)	3.77	78.9
Soyabean mixture	4.46	46.0
Poultry mixture	4.35	50.0

The coefficient equation derived from the results is :

$C = 5.47 - 0.0219 * CP \quad R^2 = 93.9\ \%$,

and since energy is given by

$$\text{Energy} = \frac{\text{ml } K_2Cr_2O_7}{C} \text{ KJ,}$$ values of energy content were

estimated for mixtures, raw materials and faeces.

Chromic oxide measurements

The chromic oxide determination in feeds and faeces was performed by a modification of the method described by Furukawa and Tsukahara (1966). During the procedure 90 - 100 mg of ground sample were weighed into a 100 ml digestion tube; to which 3 ml of oxidizing agent was added. The oxidising agent was prepared by dissolving 10g Sodium Molybdate in 150ml distilled water to which 150ml concentrated Sulphuric acid was added. The samples were then digested at 220^{o} C until they changed colour from green to yellow. Once coo,l 1 ml of hyperchloric acid was added and the solution was heated for a further 5 minutes at the same temperature. On cooling, the solution was diluted to 25 ml and centrifuged at 1500 rpm. for 10 minutes. The total concentration of chromium ion present was determined by a spectrophotometer set at 410 nm. A series of standard mixtures of known Cr_2O_3 concentration

were used in order to prepare a calibration curve by which the percentage content of the marker was estimated in all samples.

Calculation of digestibility coefficients

The nutrient digestibility coefficient was determined for each diet tested using the following equation (Hardy, 1989):

$$DC = 100 - \left[\frac{\%Cr_2O_3 \text{ in diet}}{\%Cr_2O_3 \text{ in faeces}} * \frac{\%\text{Nut. in faeces}}{\%\text{Nut in diet}} * 100 \right]$$

where nutrient is either protein, fat, carbohydrate or energy. Using the above formula, however, the digestibility coefficients for the complete diet was estimated and because the aim of this study is to evaluate the coefficient of the raw ingredients another formula has been employed:

DC(mixt)*N(mixt)*0.4895+DC(ingr)*N(ingr)*0.4895=
=DC(diet)*N(diet)

where,
DC(mixt): Digestibility coefficient of standard mixture (40%

fishmeal, 60% wheatmeal)

N(mixt) : Nutrient content of standard mixture (%)

DC(ingr): Digestibility coefficient of ingredient tested

N(ingr) : Nutrient content of ingredient tested (%)

DC(diet): Digestibility coefficient of complete diet (50% standard mixture, 50% test ingredient)

N(diet) : Nutrient content of diet (%)

0.4895: Inclusion level of standard mixture and ingredient tested, the remaining 0.021 being minerals, vitamins, Cr_2O_3 and additives.

The formula is solved for DC(ingr) and thus digestibility coefficients for protein, fat, carbohydrates and energy have been estimated for all the raw materials tested.
It is assumed that little or no nutrient interaction exists between the dietary ingredients in the test mixtures.

2.4. RESULTS

Apparent protein digestibility coefficients (ADC) for the diets containing the test ingredients and of the ingredients are displayed in Table 9. The values ranged from 45-94% for the mixed diets and from 20-95% for the respective raw ingredients. The lowest protein digestibility coefficients were obtained using feather meal, in the animal byproduct group, and tomato pulp for the plant byproduct range. All the remaining raw ingredients performed well with values greater than 60%, with the exception of meat and bone (E) and the meat and bone (L). The highest coefficient was obtained using denatured milk with a value of 95.56%. However, corn gluten meal and soybean meal gave results which were only slightly inferior.

Daily estimations showed a fluctuation of coefficient values over the experimental period (Figures 6-9).
A correlation between the *in vivo* and *in vitro* was attempted and a significant correlation between the two methods was found ($r=0.918$, $p<0.05$) and according to this finding the *in vitro* method could prove a relatively inexpensive and fast method that can be used as a first indication of protein quality.

Digestibility of lipids for both the complete diets and the raw materials are shown in Table 10. Diet digestibility

Table 9. Apparent protein digestibility (DCp) of certain raw materials and feeds by gilthead sea bream and by an industrial *in vitro* method.

BY-PRODUCT	TEST FEED MIXTURE			TEST RAW MATERIALS		
	Protein Content (%as fed)	Bream DCp (%)	*In Vitro* DCp (%)	Protein Content (%as fed)	Bream DCp (%)	*In Vitro* DCp (%)
Control	38.20	89.91	89.00	-	-	-
Feather meal	54.60	45.37	50.00	82.13	24.92	45.00
Meat and bone (C)	43.51	79.36	83.40	59.35	72.20	94.90
Meat and bone (L)	45.82	63.15	-	46.00	43.83	-
Meat and bone (E)	43.93	62.05	-	59.00	35.42	-
Lipromel	49.91	72.50	-	55.24	65.65	-
Poultry meat meal (PMM)	52.25	89.75	-	65.27	89.96	-
Poultry byproduct meal	43.89	85.28	80.00	51.65	81.89	83.00
Herring meal	53.83	92.05	-	68.16	95.84	-
Cottonseed meal	33.60	83.00	84.00	43.74	75.45	80.00
Sunflower meal	34.56	88.74	78.70	32.10	86.20	96.44
Corn gluten meal	49.62	90.41	-	65.73	90.09	-
Tomato pulp meal	26.76	69.05	76.70	21.40	20.16	61.10
Soybean meal(Solv.ext.)	38.72	92.03	88.90	43.74	90.95	97.00
Denatured milk	29.81	94.19	66.36	31.83	95.56	95.70
Flaked maize	20.71	84.00	-	8.08	60.31	-
Full fat soya meal	33.86	82.65	-	34.42	75.70	-
Maize gluten extract	25.56	80.99	-	19.03	65.30	-

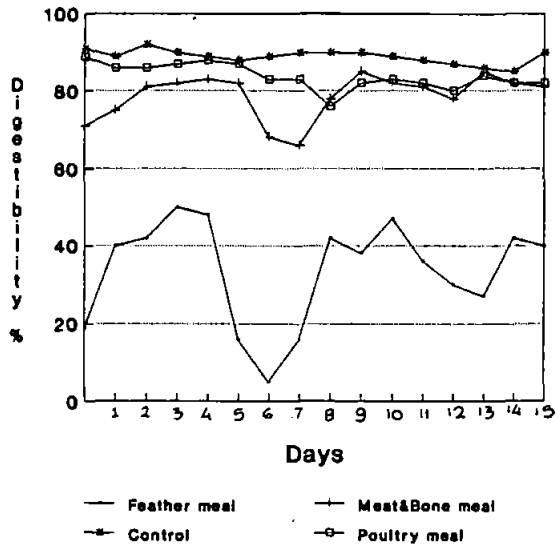

Figure 6. Variation of DCp in relation to the days of the trial.

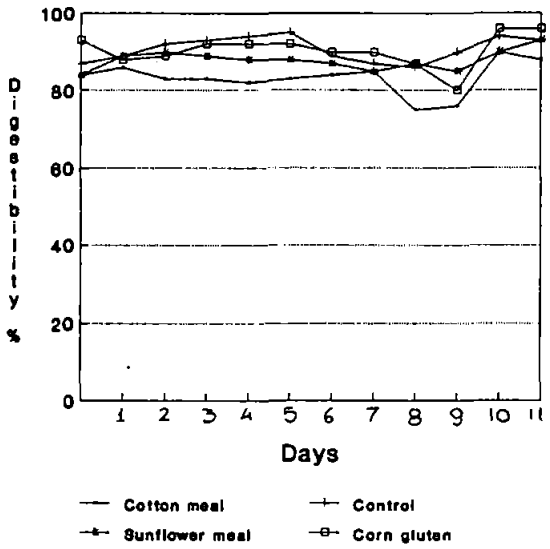

Figure 7. Variation of DCp in relation to the days of the trial.

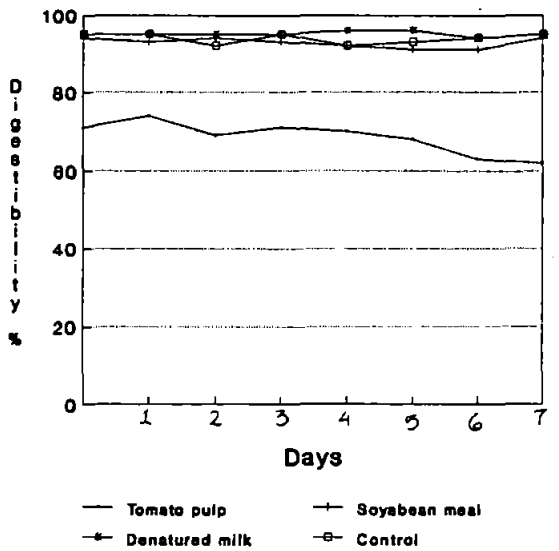

Figure 8. Variation of DCp in relation to the days of the trial.

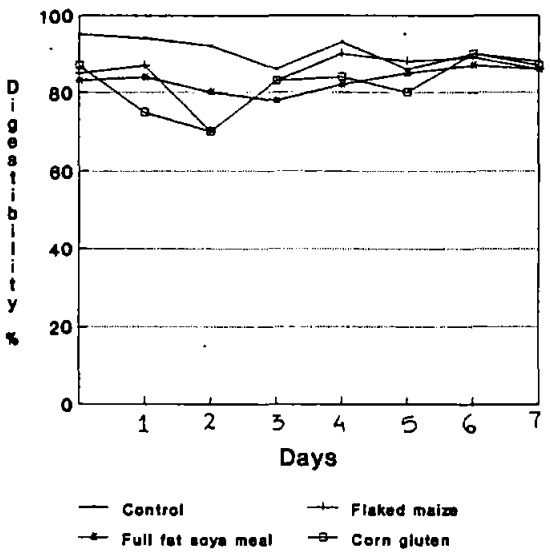

Figure 9. Variation of DCp in relation to the days of the trial.

Table 10. Digestibility (D) of carbohydrates (CHO) & lipids by seabream in test diet mixtures and respective byproducts.

	CHO Content Test feed (%as fed)	(D) CHO Test feed (%)	Lipid Test feed (%as fed)	(D) Lipid Test feed (%)	(D) Lipid Byproduct (%)
Control 1	27.74	64.26	8.40	91.60	-
Feather meal	14.21	49.72	5.80	81.60	53.68
Meat & bone meal (C)	14.32	65.73	6.79	90.90	89.78
Poultry byproduct meal	11.98	43.66	18.80	86.70	84.34
Control 2	25.21	63.90	9.60	93.80	-
Cotton seed meal	19.23	44.52	3.97	85.00	49.62
Sunflower meal	19.42	37.45	4.85	86.10	59.63
Corn gluten meal	19.09	54.42	8.14	88.30	82.95
Control 3	26.92	77.68	9.72	94.00	-
Tomato pulp	24.69	38.13	12.12	66.20	50.77
Soybean meal	22.26	59.00	4.63	87.40	62.92
Denatured milk	20.98	70.54	2.71	90.10	43.90
Control 4	25.46	56.72	9.45	91.90	-
Flaked maize	45.61	61.99	4.85	80.30	45.49
Full fat soya meal	21.35	50.66	15.63	86.60	84.65
Maize gluten extract	26.59	50.45	8.62	86.40	82.53

coefficients were all high, ranging from 66.20 for the tomato pulp containing mixture, to 90.91 for the meat and bone diet. Lipid digestibility coefficients for the raw ingredients were generally lower but still higher than those of protein. The lowest value was 43.90 for the denatured milk and the highest 89.78 for the meat and bone meal. Coefficients obtained for poultry meal, corn gluten and full fat soya were also relatively high.

Table 10 summarizes the results for carbohydrate digestibility. The fact that seabream poorly utilizes dietary carbohydrate sources, as mentioned earlier, is very profound from the coefficients estimated from this experiment. The values were generally low and ranged from 37.45 for carbohydrate in the mixed sunflower meal to 65.73 for the meat and bone (C) diet. The reproducibility, however, of the analytical method used to estimate the carbohydrate content of the faeces was not sufficient to allow more detailed comparisons to be made, and thus the values obtained for ingredient digestibility were not reliable and are therefore not presented.

Finally, energy digestibility coefficients and available energy values, ie. digestible energy (DE), are displayed in Table 11. The values ranged from 17% for the diets containing sunflower meal to 82% for the denatured milk containing mixture . More than 60% digestible energy coefficients

Table 11. Energy digestibility (D)Energy of raw materials and test feeds by seabream

	Energy content Test feed KJ/g	(D)energy Test feed (%)	Energy content Byproduct KJ/g	(D)energy Byproduct (%)	Digestible Energy KJ/g
Herring meal	20.89	77.52	22.17	94.19	20.88
Meat and bone (C)	16.42	66.96	15.70	69.27	11.37
Meat and bone (L)	17.08	52.51	17.22	32.29	5.69
Meat and bone (E)	16.59	40.36	17.11	14.65	2.51
Lipromel	22.84	55.26	26.73	34.21	9.20
Poultry meat meal	19.58	73.56	21.44	67.41	14.46
Poultry byproduct meal	22.61	73.42	24.18	80.30	18.16
Feather meal	19.32	34.48	22.29	6.77	1.31
Corn gluten meal	21.44	73.91	22.56	79.79	17.11
Sunflower meal	19.12	17.47	19.15	-36.00	-
Cotton seed meal	19.19	54.11	18.59	39.24	7.53
Denatured milk	19.09	82.84	17.09	104.32	-
Tomato pulp meal	21.39	36.45	22.10	8.83	1.89
Soybean meal (Solv.ext.)	19.06	55.69	18.81	44.79	8.54
Full fat soya meal	20.73	60.26	23.02	61.95	12.84
Maize gluten extract	19.06	42.14	18.68	23.77	6.43
Flaked maize	18.50	46.97	18.25	33.74	6.24

resulted from fish meal, meat and bone meal (C), poultry meat meal, poultry byproduct meal, corn gluten meal and full fat soya containing diets. Digestibility coefficients for the raw ingredients yielded values ranging from 6.77% for the feather meal to 94% for fishmeal. Full fat soya and meat and bone meal also performed well giving values of 61.95% and 69.27% respectively. Two unrealistic estimations of -36% for the sunflower and 104.32% for the denatured milk were calculated and this is discussed later.

2.5. DISCUSSION

Recent surveys of digestibility measurements for marine fish show that the digestibility profiles of various feeds are still rather incomplete (Cho and Slinger, 1979; Talbot, 1985; Cho et al., 1985). No previous work has been reported on the digestibility of animal and plant raw materials by the gilthead seabream.

Measurement with chromic oxide (Cr_2O_3) as an exogenous indicator (the method applied in this study) is the most frequently used method of digestion determination (Austreng, 1978; NRC, 1983; Tacon and Rodrigues, 1984), but it usually requires single feeds to be supplied in a mixture. The use of any compound as external dietary marker in digestibility studies assumes that 1) the compound is not assimilated, 2) the compound is evenly distributed in the feed, and 3) the marker moves through the digestive system at the same rate as all of the other food components (Bowen, 1978).

Recent studies have raised doubts with regard to the suitability of Cr_2O_3 as a dietary marker due to its apparent differential passage along the gastro intestinal tract (Bowen, 1978; Leavitt, 1983). However, Tacon and Rodrigues (1984) comparing the use of three external dietary markers (chromic oxide, polyethylene and acid insoluble ash) in rainbow trout (Oncorhynchus mykiss), found Cr_2O_3 to be the

most reliable in terms of reproducibility, and also polyethylene and acid insoluble ash determined coefficients were generally significantly lower. These findings seem to be contradictory to the studies of De Silva and Perera (1983) and Chandell et al. (1966) who successfully used hydrolysis resistant ash and polyethelene as markers in the Asian cichlid (Etroplus suratensis).

According to Tacon and Rodrigues (1984), the different digestibility coefficients obtained by the various methods, clearly indicate that the different physical and chemical characteristics (including density, particle size, surface area and affinity to water) may be influencing their individual flow patterns through the gastro intestinal tract with respect to digesta.

In the present study, the incorporation of Cr_2O_3 in the feed mixtures at a level of 1% was successful. However, this particular marker requires careful mixing since it tends to stick into pellets, especially under moist conditions. Poor mixing will obviously result in unrealistic estimations. There are some problems arising when an inert marker, such as Cr_2O_3, is used for digestibility studies with fish. The first major limitation is the representative collection of adequate faeces. Since the indirect approach does not call for a total quantitative recovery of faeces, it is necessary to assume that quality of faeces collected does not change with time or with nycthemeral cycle (De la Noue and Choubert,

1986). Sufficient pools and replicates are, therefore, a very important factor for realistic results. This difficulty is further increased by the possibility that nutrients will be leached from the faeces once the faeces have been voided. Nose (1960) and Inaba et al. (1962), found that estimates of digestibility in rainbow trout obtained from analyses of faeces recovered after voiding into pond water were about 10% higher than those faeces stripped manually or by dissection of the fish. Similar findings are also reported by Smith et al. (1980) and Vens -Cappell (1985). This potential underestimation of coefficients is enhanced by contamination of faeces by mucous, urinary, sexual products and undigested food residues (Cho and Slinger, 1979; Brown et al., 1985).

In the present study, faeces were collected inside a narrow tube fitted at the bottom of each conical tank, as described previously in the materials and methods section. This arrangement reduced the surface area of contact between the deposited faeces and the water and also has a number of advantages over other methods. In this respect, accurate quantitative feeding and collection is not essential, experimental fish do not have to be killed to obtain results and they can live undisturbed in normal conditions until the end of the experiment. Also a large number of fish can be included in the experiment, which will eliminate a possible effect of differences among individuals and may easily allow

the procurement of enough faecal material for subsequent analyses.

It should be noted that basic parameters such as temperature, salinity, fish size and feeding level remained fairly constant throughout the digestibility trial periods. Therefore the data obtained in the present study reflect an accurate assessment of relative values and, to a lesser extent absolute coefficients.

Evidence exists that nutrient digestibility is also slightly influenced by water temperature. However, ration level and feeding frequency may have a greater affect on the digestion rate of certain nutrient classes (NRC, 1983).

Therefore, consideration of all trial conditions is important in a comparison of different digestibility data (Brett and Groves, 1979).

Apparent crude protein digestibility coefficients (DCP) were relatively high for most feedstuffs tested (feather meal, two varieties of meat and bone meal, as well as tomato pulp produced very low coefficients) although efficiency of protein digestion was determined to be significantly higher for some ingredients than for others. These high protein coefficients indicate that dietary protein in test diets was well digested by seabream in the main, regardless of its source. There was no obvious correlation between the crude protein content of the materials and their protein digestibility.

Where comparisons are possible, it is noted that Hanley (1987) testing different feedstuffs for tilapia (Oreochromis niloticus) reported values for soybean meal (90.73%), and a lower result for poultry offal meal (73.83%). However, rainbow trout, fed similar feed formulations as the ones used by the author resulted in a higher value for feather meal (65%); for sunflower meal (94.73%) and for tomato pulp (79.15%). Coefficients obtained testing meat and bone meal, poultry meal, cotton seed meal, corn gluten meal, denatured milk from the same study, were very similar, whereas soyabean protein was digested less efficiently by rainbow trout (81.17%) (Alexis, unpublished data).

In agreement to these findings, are the values obtained by Cho and Slinger (1979) who both tested cotton seed meal, meat and bone meal, poultry meal, corn gluten, feather meal, and soyabean meal in a series of digestibility trials for rainbow trout.

In recent studies involving crustacea, Reigh et al. (1988) evaluated various feedstuffs in formulated diets for the red swamp crayfish (Procaborus Clarkii) and obtained comparable results to those obtained in the present investigation. Cotton seed meal protein was utilized more efficiently by red swamp crayfish (83.7%), but soyabean meal and meat and bone meal gave very similar coefficient values of 94.8 and 76.5% respectively. Kirchgessner et al. (1986) examined the digestibility of 23 feed mixtures for carp (Cyprinus carpio).

The most important protein components used were fishmeal, blood meal, meat and bone meal, hydrolyzed feather meal, corn gluten, Brewer's yeast, soyabean meal and various legumes. The mixes gave coefficients which ranged from 70-90%. Law (1986) also tested fishmeal, soymeal, and maize meal on grass carp (Ctenopharyngodon idela) and obtained digestibility coefficients of 90.81, 96.21 and 50.61% respectively. Brown et al. (1985) estimated apparent protein digestibility coefficients for Channel catfish (Ictalurus punctatus) using several raw materials and obtained values of 92% for corn gluten meal, 86% for soyabean meal, 82% for meat and bone meal and 65% for poultry byproduct meal.

It should be mentioned that nutrient interactions within materials can play a significant role on the nutrient digestibility. High dietary ash levels are reported, for example, to have a negative correlation with protein digestibility (Nose and Mamiya, 1963). All types of meat and bone meal tested in this study contained varying ash levels.

High levels of fibre have also been shown to interfere with the digestion and absorption of other nutrients (Buddington, 1979; Anderson, 1985). Tomato pulp meal in the present study having a high level of crude fibre exhibited very low protein digestibility which might be explained by this effect.

Processing methods can also influence the digestibility of some materials (Ogino and Chen, 1973; Smith, Kirchgessner, 1986) and this is a particularly difficult variable to define in the context of the feed industry.

The daily variability in apparent protein digestibility calculated in the present study showed a daily rhythmicity, which was not always consistent. In agreement are the findings of De Silva and Perera (1983) on Tilapia fry. These authors suggested that the observed rhythmicity in digestibility could be an apparent phenomenon manifesting the individual variability of the experimental group. Even so, this could be of significance under farmed conditions where a feeding trial is performed with a large group of fish and not on an individual basis. However, the earlier observations on the rhythmicity in tilapia fry as well as those presented here, lead us to believe that the daily rhythmicity is a true phenomenon and not an artifact related to the methodology.

Seabream exhibited the ability to efficiently digest and assimilate lipids of both animal, marine and plant origin and again there was no correlation between lipid content of the material and lipid digestibility. In close agreement are the coefficients obtained for corn gluten (89.7%), feather meal (68.0%), and poultry by product meal (83.6%) in rainbow trout (Cho and Slinger, 1979). It is well known that most species of fish are capable of efficiently digesting different

classes of dietary lipids. However, problems arise when harder saturated type fats are included in marine fish diets (Austreng et al., 1979). The latter authors reported digestibility coefficient values averaging 85% for fats fed to rainbow trout. In their experiment, unsaturated fatty acids showed higher digestibility than saturated fatty acids of the same chain length. Watanabe (1989) found that in both, carp and rainbow trout, hydrogenated oils with melting points of 53^{o} C were poorly digested. On the other hand, beef tallow and hydrogenated fish oil of melting point 38^{o} C were found to be well digested.

Carbohydrates are always considered as being the least expensive energy sources and the aim of the feed formulator is to include as much carbohydrate based material as possible in the feed to provide a protein sparing effect. However, this is not always possible as some fish, notably carnivorous species such as seabream, may have a low tolerance for dietary starches (Spannhof and Plantikow, 1983).

In this study, problems with the standardization of the chemical method for available carbohydrate produced unreliable results, and thus values are not yet presented for raw material digestibility coefficients.

Digestibility coefficient of gross energy (DCE) in the experimental ingredients depend on the relative digestion of proteins, fats and carbohydrates, and as a result values do

not necessarily show the same trends as protein and fat digestibility. The gross energy digestibility of the diets ranged from 6.77% to 80.30%. Apparent energy digestibility coefficients indicated that materials high in carbohydrates resulted in lower energy digestibility values. However, denatured milk produced a satisfactory result. Except for feather meal, and high ash meat and bone meals all animal byproducts and also corn gluten and full fat soya showed good energy digestibility values. By comparison with work in tilapia (O. niloticus), Hanley (1987) found a higher result for soybean meal (56.5%). According to Wilson and Poe (1985), meat and bone meal was 70-76% digested by channel catfish (Ictalurus punctatus), a similar value to that found in this study. Cho and Slinger (1979) estimated energy digestibility for a series of feedstuffs for rainbow trout. The values they obtained were 81.2% for corn gluten, 73.7% for feather meal, 74.7% for poultry byproduct meal and 82.3% for soyabean meal.

The negative digestibility coefficient estimated for sunflower meal has been recorded for fish by other workers (Davies, 1985). It is suggested that high fibre contents of some feedstuffs lead to errorous results. Whilst for milk, the energy digestibility value of 104.32 % indicates that this raw material is completely digestible in terms of gross energy and that a slight overestimation may occur due to an artefact of the method employed for chromic oxide

determination.

In conclusion, the results indicate that protein and fat digestibility was relatively high for most materials tested. Choice of protein supplements, therefore should depend on protein content and amino acid balance and availability, since apparent protein digestibility coefficients for most of the tested supplements are adequate.

On the basis of the parameters reported, a selection of the most promising animal and plant byproducts were then used in a series of growth trial evaluations by either the partial or complete substitution of fishmeal as the reference protein source.

CHAPTER 3

GROWTH TRIALS TO EVALUATE ANIMAL PRODUCTS AND BYPRODUCTS IN BALANCED TEST DIETS FOR THE GILTHEAD SEABREAM (SPARUS AURATA)

3.1. INTRODUCTION

The main contribution of any dietary protein is to satisfy the essential amino acids (EAA) required by the animal for the maintenance and growth of different tissues (Ash, 1985; Kaushik , 1989). Recent research on teleosts has shown that the relative contribution of dietary EAA to the overall whole body amino acid pool is much greater in the case of fish compared to higher terrestrial animals (Fauconneau, 1985). This implies that more attention should be paid to the constraints regarding the balance and availability of all the dietary essential amino acids.

Fishmeal produced from good quality whole fish which has been properly processed is the highest quality protein source commonly available to fish feed manufacturers (Reinitz and Hitzel, 1980; Lovell, 1989). Fishmeal usually contains 60 to 80% protein which is 80 to 95% digestible for most fish species (Cho and Slinger, 1979; Hanley, 1987). It is relatively high in lysine and methionine and also contains essential fatty acids of the n-3 series. Besides providing protein and EAA, fishmeals are potentially important sources

of minerals, and the availability of such essential nutrients can vary depending on the origin and processing of the meal (Kaushik, 1989).

The assessment of the potential nutritional value of any new protein source should be made against a standard high quality fishmeal whose nutritional value is well established and defined (Watanabe et al., 1983).

The unique value of feeds of animal origin in upgrading the nutritional qualities of diets for monogastric animals is well recognized (Gropp et al., 1979; Koops et al., 1982). In diets based on plant products, it is often difficult to avoid a deficiency in essential amino acids and some important vitamins (Tacon et al., 1984; Wilson and Poe, 1985a). Feeds of animal origin can usually supply the correct amino acids and vitamins and for this reason animal products, even when used in small amounts, can improve the nutritional value of the diet (Jauncey and Ross, 1982). As a group, however, the feeds of this category are deficient in the sulphur containing amino acids, methionine and cysteine (Harris, 1978; Hardy, 1989). Another characteristic of this group of products is their high ash content, especially with respect to calcium and phosphorus content. These high levels are due to the presence of appreciable amounts of bone and mineralized tissue. Their fat content tends to vary widely and is generally easily oxidized and hence complicates the storage properties of finished diets containing these

materials (Dosanjh et al., 1984).

Animal feed ingredients are often byproducts of food processing industries and are obtained when high value food for humans is extracted from a raw material. Once the high value products have been removed, the remaining material is further processed to produce a material that itself becomes an article of commerce (ADCP, 1983). These ingredients are normally available throughout the year with prices depending on supply and demand. Animal byproducts are derived from the meat-packing, poultry-processing and rendering industries. The protein content of these products after drying ranges from 50% to over 85% and these are established standards for the quality of these proteins. The essential amino acid composition of animal byproduct meals is similar to that of whole-egg protein, which is the standard by which protein quality is judged (Sabaut and Luquet, 1973). These meals are good sources of lysine but poor sources of methionine and cysteine, which are usually found to be limiting in diet formulations (Hardy, 1989).

Animal meat meals and meat and bone meals are the principal byproducts of abbatoirs and slaughterhouses. They include dried mammalian tissues, exclusive of hair, hooves, horn, manure and stomach contents. The protein content of these meals is about 50 %, while their fat content averages 10%. The principal difference of the two products is the higher levels of phosphorus (P) and calcium (Ca) of the meat

and bone meal. Both ingredients have relatively high ash contents averaging 30% of the dry matter.

Blood meal is characteristically defined as the resulting dry product from clean, fresh animal blood, exclusive of all extraneous materials. It is usually spray dried, flash dried or conventionally dried in a cooker. Blood meals have a very high protein level of about 85%, are rich sources of lysine but are deficient in methionine.

Poultry by product meals are also valuable materials obtained from the wastes of the large commercial poultry processing plants. It is usually exclusive of feathers and intestines but may contain the feet and head in association with rendered meat from the carcass. These byproduct meals vary in composition depending on the processing applied and the materials that are included in the meal. In all cases, however, the degree of heat treatment used during drying and sterilization is of considerable importance in relation to the nutritional value and this will be discussed later.

Hydrolyzed poultry feather meal is made from feathers which have been hydrolyzed under pressure in the presence of calcium hydroxide; $Ca(OH)_2$ and dried. It has a high protein content but is deficient in some of the essential amino acids. Its protein is predominantly Keratin which is very rich in cysteine.

Finally, several dairy byproducts are used as ingredients in fish diets. These include milk whey, casein and dry skim

milk. The protein content of whey products is usually low (13-17%). Dried skim milk has a very high biological value, is highly digested and has an excellent amino acid profile with a protein content of about 34% (Cieslak et al., 1986).

A variety of the above mentioned animal byproduct meals have been tested for use in commercial fish feeds with variable success. These include, poultry byproducts (Higgs et al., 1979; Chan et al., 1981; Alexis et al., 1985; Steffens, 1987), meat and bone meals (Fowler and Banks, 1976; Tacon et al., 1983a; Davies et al., 1989), hydrolyzed feathermeal (Fishelson and Yaron, 1983), blood meal (Reece et al., 1975; Asgard and Austreng, 1986; Otubusin, 1987), egg processing wastes (Davies et al., 1976) and earthworm powder (Stafford and Tacon, 1985) besides many more.

Gilthead seabream is a carnivorous species and thus animal byproducts would be the ideal first choice for experimentation. Two sets of experiments were designed to evaluate differently processed meat and bone meals at varying dietary inclusion. Also the feasibility of using different poultry and feather meals was tested from low substitution levels to complete fishmeal replacement.

3.2. EXPERIMENT 2

PRELIMINARY EVALUATION OF DIFFERENT MEAT MEALS AND RELATED BYPRODUCTS FOR SEABREAM

3.2.1. ABSTRACT

Seabream (Sparus aurata) fingerlings having an initial mean weight of 4.5g were fed a series of experimental diets containing various inclusions of differently processed meat and bone meals and a high fat animal and poultry blend. Three types of meat and bone byproducts were used to substitute 20 and 40% of the fish meal protein component. A commercial product, Lipromel$_R$ was used at a level of 15% of the dietary protein, limited by its high fat content. The diets were formulated to be isonitrogenous and isocalorific with respect to gross protein and energy, (ie. 50% crude protein, 10% crude fat and 18 MJ/Kg).

After a 72 day feeding trial, all the diets performed as well as the control fishmeal based formulation. However one meat and bone meal source produced slightly better results at a 40% inclusion level. Feed efficiency (FE) ranged from 65.8 to 62.5%. Specific growth rates (SGR), protein efficiency ratios (PER) and net protein utilization (NPU) values were similar for all diets. It was concluded that meat and bone meal can effectively replace up to 40% of the fishmeal protein component in diets for bream.

3.2.2. MATERIALS AND METHODS

3.2.2.1. Experimental fish and feeding regime

Gilthead seabream (Sparus aurata) fingerlings having a mean weight of 4.6g were obtained from the Cefalonia Fisheries hatchery situated on the island of Cefalonia in Greece. After transportation by air to Polytechnic South West, the fish were treated with a medicated diet containing oxytetracyclin as a prophylactic measure. The stock was then acclimated to the new environmental conditions for one month and then randomly distributed to each of the six experimental tanks for the subsequent feeding trial. The stocking density was decided to be thirty fish per tank and the remaining fish were sacrificed by lethal anaesthesia with benzocaine and analyzed for initial gross carcass composition. Fish were fed the experimental diets six days per week at a level of three per cent of the body weight per day in four discrete meals. Following the one day of starvation, every week, the fish were weighed and the feeding rate recalculated. This protocol was maintained for the 72 day growth trial period. On termination of the experiment, the fish were weighed individually and killed for gross chemical analysis and routine histopathological examination.

3.2.2.2. Experimental facilities

The experiment was conducted in a recirculatory sea water system employing a large biological filtration unit and six self-cleaning experimental tanks of 500 litre capacity situated within the basement aquarium of the Fish Nutrition Unit (Plate 4). Each tank received a parallel input of water at a flow rate of 2.5 l/min. The temperature was held automatically at 22^{o} C. pH maintained at 7.8 by the addition of Sodium Carbonate when required. Photoperiod was set to operate on a 12hrs light/12hrs dark cycle using artificial illumination from fluorescent tubes simulating natural daylight. The water quality was also monitored routinely for dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3). A tanker of fresh seawater was supplied to the facility on a monthly schedule and was used for weekly partial replenishment of the closed recirculation system. A freshwater inlet valve was connected to the system to compensate for evaporation losses, thus allowing on effective control of salinity within 33 - 36ppt. All water quality parameters appeared to be within the known tolerance limits for the gilthead seabream under similar conditions.

3.2.2.3. Experimental diets

A series of experimental diets was designed to evaluate

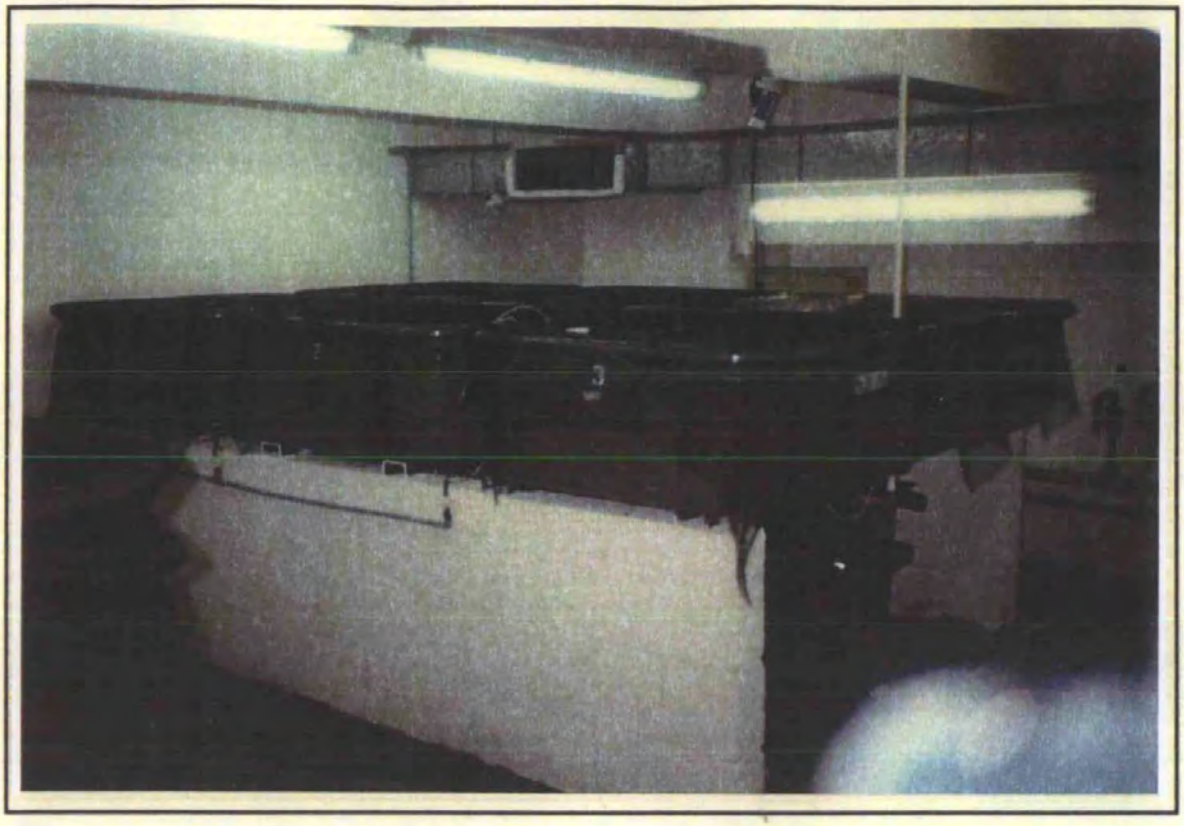

Plate 4. Large recirculation units for growth studies situated at Polytechnic South West, Plymouth.

several meat and bone meal type products as partial ingredients in diets for seabream. Apart from the conventionally processed meat and bone meal (MBC), two more refined products were tested, together with a commercial meat / fat blended product; these were:

1. The meat and bone (London source) (MBL) was a high quality raw material from potentially edible raw materials. It originated from selected butchery wastes and meat processing factories, with some sources from abattoirs. It was comprised entirely of residual clean fat and bone and has a high mineral content. The material was cooked under vacuum at 105^{o}C for one hour.

2. The meat and bone (Exeter source) (MBE), was produced from raw materials of ruminant and pig origin only. It was cooked at 104-106^{o} C for two hours.

3. The meat and bone (MBC), was obtained from a locally based large abattoir at Torrington, Devon. It is a standard grade product derived from slaughterhouse waste and is variable in quality.

4. A high fat blend of enzymatically hydrolyzed animal proteins and emulsified fats (Lipromel$_{R}$) was also used to replace 15% of the fish meal protein. The main constituents of Lipromel$_{R}$ are processed blood, feather meal and poultry offal and these are processed by heating (101^{o} C) the product for eight hours.

The proximate analysis of the raw materials and test diets

are displayed in Tables 12 and 13.

The composition of the test diet formulations and their respective amino acid profiles are shown in Tables 13 and 14.

MBC meal replaced white fishmeal (provimi-66) at two substitution levels (20,40%) whereas MBL and MBE both substituted 40% of the total protein level. A control diet was also formulated consisting of white fishmeal as the principal protein source.

Each of the experimental diets were designed to be isonitrogenous (50% crude protein) and isocalorific (18.5 MJ/Kg GE). Lipid levels were set at 10% by addition of cod liver oil where necessary, and the carbohydrate content was adjusted to 8% of the diet using both corn starch and dextrin in a ratio of 2:1.
Separate mineral and vitamin premixes were also included to satisfy the requirements for this species (Table 15).

3.2.2.4. Analytical techniques

Protein contents (N*6.25) of the raw materials, the formulated diets and fish samples were determined by the Kjeldahl method (AOAC, 1984). Amino acid profiles for diets have been determined by Eurolysine Ltd in France. The procedure involved a preliminary acid hydrolysis of the samples in 6N HCl, followed by Ion-exchange chromatography (Beckman 6300-amino acid analyzer).

Table 12. Nutrient composition of raw materials tested.

	(% as recieved)						
	Moisture	Protein	Lipid	Ash	Fibre	NFE	Available Lysine (% protein)
White fish meal	2.82	66.18	8.63	21.12	2.55	-	6.54
Poultry meal (PM)	9.35	53.66	30.20	5.80	1.98	-	2.88
Poultry meal defatted (DPM)	11.22	68.10	8.24	6.96	2.38	-	-
Poultry meat meal (PMM)	5.00	62.00	13.50	15.00	2.00	-	5.43
Meat & Bone (C)	2.46	59.35	8.90	24.84	5.13	-	3.54
Meat & Bone (E)	5.00	49.00	10.00	32.00	2.00	-	3.75
Meat & Bone (L)	7.00	46.00	12.50	33.00	2.00	-	4.29
Lipromel	10.00	55.24	30.00	8.00	2.00	-	3.78
Feather meal	10.67	77.59	11.90	2.01	-	-	1.66
Poultry meal 1 (PM1)	10.37	61.05	25.17	3.45	1.09	-	-

NFE calculated by difference

Table 13: Formulation of experimental diets for seabream using various meat and bone meals and related products showing percentage replacement of dietary protein.

	DIET 1 FM 100%	DIET 2 MBC 20%	DIET 3 MBC 40%	DIET 4 MBE 40%	DIET 5 MBL 40%	DIET 6 LIP 15%
White fish meal	74.01	62.33	46.74	46.74	46.74	66.22
Meat and bone (C)	-	16.46	32.93	-	-	-
Meat and bone (E)	-	-	-	39.36	-	-
Meat and bone (L)	-	-	-	-	42.51	-
Lipromel	-	-	-	-	-	14.17
Cod liver oil	3.61	3.17	3.04	1.77	0.65	-
Starch/Dextrin	8.00	8.00	8.00	8.00	8.00	8.00
Ascorbic acid	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.40	0.40	0.40	0.40	0.40	0.40
Mineral premix	0.70	0.70	0.70	0.70	0.70	0.70
a-cellulose	12.28	7.94	7.19	1.43	-	8.98
Binder (guar gum)	0.40	0.40	0.40	0.40	0.40	0.40
NUTRIENT ANALYSIS (%)						
Moisture	5.85	4.12	4.18	5.11	6.37	7.55
Protein	47.22	49.70	49.22	48.85	49.01	50.10
Lipid	9.24	9.35	9.17	9.55	9.16	9.25
Ash	17.01	18.15	19.15	21.59	26.03	15.70
Fibre	13.47	9.90	8.64	4.69	1.16	9.88
NFE	7.21	8.78	9.70	10.21	8.27	7.52
Energy (MJ/Kg)	18.91	19.23	19.42	17.14	17.09	18.78

Nitrogen Free Extracts (NFE) calculated by difference

Table 14. Amino acid profile of diets containing different meat and meat and bone meal ingredients (% of diet).

	FM 100%	MBC 20%	MBC 40%	MBE 40%	MBL 40%	LIP 15%
Aspartic acid	4.75	3.97	4.39	4.33	4.30	4.20
Threonine	1.94	1.77	2.11	2.03	2.09	1.95
Serine	2.32	2.22	2.62	2.52	2.42	2.45
Glutamic acid	6.22	6.03	6.33	6.20	6.45	6.39
Glycine	5.03	5.38	4.10	4.23	4.78	4.94
Alanine	3.06	3.15	2.27	3.02	3.14	3.11
Cysteine	0.41	0.33	0.47	0.36	0.39	0.49
Valine	2.14	2.04	2.46	2.11	2.12	2.18
Methionine	1.19	1.12	1.35	1.54	1.12	1.19
Isoleucine	1.72	1.63	1.92	1.89	1.78	1.70
Leucine	3.06	2.92	3.51	3.00	3.10	3.04
Tyrosine	0.50	1.33	-	1.18	1.14	1.01
Phenylalanine	1.60	1.64	1.92	1.71	1.75	1.66
Histidine	0.97	0.95	1.13	1.02	0.98	0.95
Lysine	3.05	3.02	3.37	3.30	3.30	2.97
Arginine	3.54	3.56	3.39	3.42	3.44	3.56
Tryptophan	0.38	0.35	0.42	0.42	0.40	0.36

Table 15. Vitamin and mineral premix formulations used for the experimental diets for seabream (Supplied by Redmills, Kilkenny, Rep. Ireland).

Vitamins	(g)/Kg
Vitamin A (1000 10/g) (acetate or palmitate)	800.00
Vitamin D3 (1000 10/g)	50.00
Vitamin E (1000 10/g)	15.00
Vitamin K (menadione sodium bisulphate)	2.70
Thiamin HCl	5.00
Riboflavin	4.00
D-Calcium pantothenate	15.00
Biotin	0.05
Folic acid	1.00
Vitamin B12 (0.1%)	3.00
Niacin	25.00
Pyridoxine HCl	3.00
Ethoxyquin	8.00
Wheat middlings to make 1 Kg	68.25
Minerals	
Iodized salt (99% NaCl, 0.015% I)	300.00
Potassium Iodide (KI) (76% I)	1.00
Manganous Sulphate ($MnSO_4H_2O$)	35.00
Ferrous Sulphate ($FeSO_4$ $7H_2O$) (21% Fe)	30.00
Copper Sulphate (Cu_2SO_4 $5H_2O$) (25% Cu)	10.00
Zinc Sulphate ($ZnSO_4H_2O$) (36% Zn)	40.00
Wheat middlings to make 1 Kg	316.00

Lipid content was assayed by soxhlet extraction using petroleum ether (b.pt. 40-60^{o} C). Moisture content was determined by oven drying all samples at 105^{o} C for 24 hrs, ash content by incineration in a muffle furnace at 550^{o} C for 12 hrs and fibre content according to Weende method outlined in AOAC (1984).

Gross energy was estimated by the wet oxidation method described by O'Shea and Maguire (1962) with some slight modification as before (section 2.3.5.).

" Available" lysine was determined for all the products and fishmeal using the FDNB "available" lysine method described by Booth (1971). The method is a modification of the Carpenter (1960) method and is based on the assumption that lysine which does not react with FDNB is not nutritionally available even though it would be included in the conventional measurement of total lysine in acid hydrolysates. FDNB reacts with the E-NH_2 groups of lysine in a protein. Each free amino acid becomes labelled with a dinitrophenyl (DNP) group that resists subsequent digestion with acid, so that each N-terminal amino acid unit that had originally reacted is converted to an a-DNP-amino acid. These are yellow in colour and are separated and identified by spectrophotometry. The method is summarized below:

Available Lysine

Half a gram of accurately weighed sample, ground to pass through a 0.5mm sieve, was placed in a round bottomed flask. 10 ml of $NaHCO_3$ solution (80g in 1l distilled water) were added and the flask was gently shaken and left to stand until the sample was wetted. 15 ml of the FDNB solution (0.4 ml FDNB in 15 ml ethanol) was added to each flask. The flasks were stoppered and shaken for at least 2 hours. The ethanol was then evaporated on a boiling water bath. After cooling 30 ml of 8.1 M HCl was added and refluxed gently for 16 hours. The contents were then filtered, while still hot, into a 250 ml volumetric flask. The digestion flask and the residue were washed thoroughly with water until the total volume of the filtrate was almost 250 ml. When the filtrate had cooled it was made to volume (250 ml) and mixed. 2 ml of the filtrate was pipetted into each of two test tubes labelled A and B. The contents of tube B were extracted with 5 ml peroxide free diethyl ether. As much of the ether was discarded and the tubes were placed in hot water until effervescence from the residual ether had ceased, and left to cool. A drop of phenolphthalein solution (400mg/l of 60% ethanol) was added and then NaOH solution (120g/l) from a dropping pipette until the first pink appeared. 2ml of carbonate buffer (pH 8.5) was then added. Under the fume hood, 5 drops of MCC was added, the tubes firmly stoppered and shaken vigorously. After about 8 minutes, 0.75 ml of

concentrated HCl were added cautiously. The solution was extracted with ether as described previously but repeated four times. The tube was then cooled and the contents made up to 10 ml with distilled water. Tubes A were extracted three times with ether as described before and the contents made up to 10 ml with 1 M HCl. The absorbance of both A and B were measured at 435 nm. Reading A minus reading B (the blank) is the net absorbance attributable to DNP-L. A Standard solution of DNP-L was also pipetted into two tubes A and B and the previously described procedure repeated. The available lysine was then calculated using the formula:

$$C = \frac{Ws * V * 100 * 100}{Wu * As * a * CP}$$

where,

C : Available lysine content as g lysine/16g N

Ws : Weight of standard, expressed as mg lysine/2ml

Wu : Weight of sample (mg)

As : Absorbance of the standard

Au : Absorbance of the sample

V : Volume of filtered hydrolysates (ml)

a : Aliquot of filtrate (ml)

CP : Crude protein content.

Histological examinations

Standard histological procedures were undertaken on selected organs and tissues at the end of the experiment. The sections were stained with heamatoxylin and eosin and examined for any pathological symptoms.

Statistical analysis

Statistical interpretations were made using analysis of variance at the 5% level of significance. Estimations were made using the Minitab utility of the main frame of Polytechnic South West. Duncans multiple range test (Duncans, 1955) was also applied to mean values where appropriate.

3.2.3. RESULTS

All the fish became accustomed to the new feeds very soon and showed no palatability problems. The growth parameters, feed utilization and carcass composition details are summarized in Table 16. Mean final weight did not differ significantly among the fish fed all the diets (Figure 10). A slight difference, however, was observed with the group feeding on the 40% MBL diet, where the fish performed better. Specific growth rate and percentage weight gain followed the same trend.

Feed efficiencies were within acceptable limits and ranged from 65.79% for the 40% MBE diet, to 62.50% for the Lipromel formulation. Protein efficiency ratios and net protein utilizations were similar for all treatments but the group fed the Lipromel diet yielded lower values, indicating a possible lower protein efficiency and reduced body nitrogen deposition for this tested material.

Diets containing 20% MBC, 40% MBL and 15% Lipromel resulted in a significantly higher ($p<0.05$) protein content in the whole seabream carcass compared to the control group. Although the percentage of protein in the fish increased in all of the treatments throughout the trial period, there was no significant differences ($p<0.05$) in the final moisture, fat and ash contents of fish fed the respective diets.

The histological examination of seabream at the end of the

Table 16. Growth performance and feed utilization of seabream fed experimental diets containing various meat and bone meals.

	DIETS						
	FM 100%	MBC20%	MBC40%	MBE40%	MBL40%	LIP15%	+SEM[1]
Initial mean weight (g)	4.51[a]	4.48[a]	4.57[a]	4.53[a]	4.66[a]	4.67[a]	0.11
Final mean weight (g)	20.61[a]	19.92[a]	20.80[a]	20.34[a]	22.06[a]	20.99[a]	0.57
Weight gain (%)	356.98	344.64	355.14	349.01	373.39	349.46	
Specific growth rate (%/day)	1.81	1.78	1.80	1.79	1.85	1.79	
Food intake (mg/day)	353.30	334.53	344.89	333.76	369.76	362.67	
Weight gain (mg/day)	223.61	214.44	225.42	219.58	241.67	226.67	
Feed efficiency (%)	63.29	64.10	53.36	65.79	65.36	32.50	
Protein efficiency ratio	1.26	1.23	1.27	1.27	1.25	1.16	
Nitrogen intake (mg/day)	166.83	166.26	169.75	163.04	181.22	181.70	
Nitrogen deposition (mg/day)	37.01	36.00	37.24	36.25	40.89	37.97	
Apparent net protein utilization	22.18	21.65	21.94	22.23	22.56	20.90	
CARCASS ANALYSIS (% wet weight)							
	Initial Fish						
Moisture	68.99	68.42[a]	68.61[a]	69.93[a]	68.68[a]	69.27[a]	69.52[a]	0.40
Protein	15.12	16.55[a]	16.79[ab]	16.52[a]	16.51[a]	16.92[b]	16.75[b]	0.23
Lipid	9.31	9.38[a]	9.10[a]	8.43[a]	9.13[a]	8.76[a]	8.74[a]	0.29
Ash	3.98	3.95[a]	4.03[a]	4.02[a]	4.06[a]	4.01[a]	3.99[a]	0.09

Figures with common superscripts in each horizontal row are not significantly different ($p<0.05$)

[1] SEM Standard Error of the Mean calculated from residual mean square in the analysis of variance.

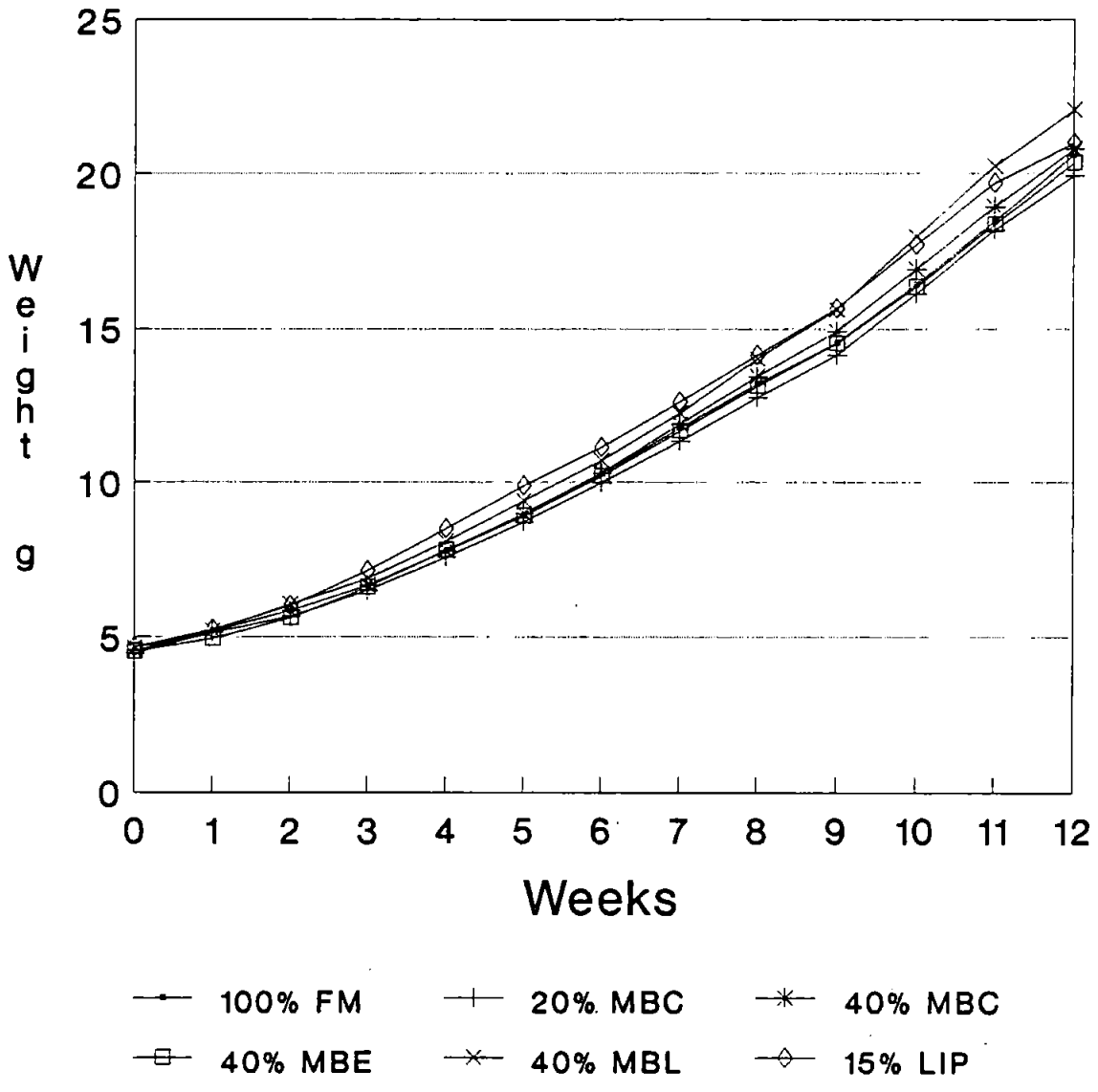

Figure 10. Mean weight increase of sea bream fed diets containing various meat and meat and bone meals.

experiment showed no diet related pathological symptoms and the fish appeared quite normal.

The use of meat and bone meal for seabream was proved successful for all the material varieties and for all the inclusion levels contributing 20 and 40% of the total protein component.

Different processing technology does not appear to have any effect in the current trial applied to animal meat products on the performance of the experimental diets. The standard grade meat and bone meal product gave the same results as the more expensive MBE and MBL materials, and the "available" lysine values obtained were relatively similar for all products.

The high protein, fat and energy digestibilities estimated for meat and bone meal in previous experiments may help to explain the high growth and feed utilization values obtained in this study.

A higher level of substitution of meat and bone meal to fish meal will be interesting, and further studies were carried out in this direction.

3.3. EXPERIMENT 3

FURTHER SUBSTITUTION OF FISHMEAL WITH A COMMERCIAL STANDARD MEAT AND BONE MEAL

3.3.1. ABSTRACT

In continuation of the previous experimental objective, the feasibility of further substitution of fishmeal with meat and bone meal was tested. The conventionally treated meat and bone meal (MBC) was used because of the encouraging results it yielded and because it is more typical of commercially available meat and bone meal on the market.

Juvenile gilthead seabream of mean weight of 15g were used in the feeding trial for 56 days, using a daily fixed feeding rate of 3% of body weight.

The meat and bone meal was substituted at levels of 40, 60, 80 and 100% of the protein in the diet. A white fishmeal based control formulation as well as a commercial marine fish diet were used in the trial as references. All the diets were designed to be isonitrogenous (CP:50%) and isocalorific in gross energy having a lipid content of 12% on an as fed basis.

There was a significant reduction in all growth performance parameters for the diets containing more than 40% meat and bone meal. Feed efficiency ranged from 61% for the

commercial diet to 22.5% for the group fed 100% MBC. Specific growth rates (SGR), Protein efficiency ratios (PER) and apparent net protein utilization (NPU) followed the same trends. A noticeable reduction of the carcass lipid content was observed at the higher MBC substitution levels.

3.3.2. MATERIALS AND METHODS

3.3.2.1. Experimental fish and feeding regime

Sparus aurata fingerlings were graded into six groups of 12, having an initial mean weight of 15g. The fish again originated from the Greek hatchery in Cefalonia and were transported to Polytechnic South West by air and treated the same way as described in the previous section.

The fish were fed for 56 days at a fixed feeding level of 3% of body weight daily. The total biomass of each tank was measured in biweekly intervals to adjust the feeding level accordingly.

A number of representative fish were sacrificed at the beginning and on termination of the experiment and were analyzed for gross chemical analysis as well as routine histological examination.

3.3.2.2. Experimental facilities

The holding facility comprised of a six 30l tank closed recirculation sea water system with a 300l biofiltration unit (Plate 5). The experimental unit was situated at the basement aquarium of the Polytechnic South West. Water supply and quality as well as experimental conditions were monitored and maintained at similar levels as described in section 3.2.2.2..

Plate 5. Small scale unit for short term feeding trials.

3.3.2.3. Experimental diets

Table 17, presents the formulation and nutrient composition of the tested mixtures; amino acid profiles are shown in Table 18. Four of the experimental diets were designed to test higher substitution levels of meat and bone meal in diets for seabream. These were prepared by replacing 40, 60, 80 and 100% of the fishmeal protein component, with meat and bone meal (MBC). Two reference diets were also employed, a 100% fishmeal mixture and a commercial marine fish diet (Aqualim, France).

The diets were designed to be isonitrogenous at 50% crude protein content, isocalorific at 18 MJ/Kg of gross energy and to have a lipid level of 12% on an as fed basis. Addition of marine oil, vitamins, minerals, starch, dextrin, a-cellulose and binder was used to balance the diet as displayed in Table 17.

3.3.2.4. Analytical techniques

All the same analytical procedures were followed in this present study as described in previous sections (section 3.2.2.4.)

Table 17. Formulation of experimental diets for seabream using further inclusion levels of meat and bone meal.

	DIET 1 FM 100%	DIET 2 MBC 40%	DIET 3 MBC 60%	DIET 4 MBC 80%	DIET 5 MBC100%	DIET 6 COMERCIAL[1]
White fish meal	80.00	48.00	32.00	16.00	-	*
Meat and bone (C)	-	33.70	50.55	67.40	84.25	*
Cod liver oil	6.41	5.74	5.26	4.88	4.40	*
Starch/Dextrin	8.00	8.00	8.00	8.00	8.00	*
Ascorbic acid	0.10	0.10	0.10	0.10	0.10	*
Choline chloride	0.50	0.50	0.50	0.50	0.50	*
Vitamin premix	0.40	0.40	0.40	0.40	0.40	*
Mineral premix	0.70	0.70	0.70	0.70	0.70	*
α-cellulose	3.49	2.46	2.09	1.62	1.15	*
Binder (guar gum)	0.40	0.40	0.40	0.40	0.40	*
NUTRIENT ANALYSIS (%)						
Moisture	5.45	6.16	7.49	6.28	6.61	5.92
Protein	49.25	47.32	47.12	47.76	46.10	47.58
Lipid	12.67	12.28	11.80	11.85	11.41	11.55
Ash	18.01	19.35	19.30	21.02	20.95	11.19
NFE	14.62	14.89	14.29	13.09	14.93	23.76
Energy (MJ/Kg)	19.91	19.43	18.65	18.19	19.09	20.2

NFE, Nitrogen Free Extracts calculated by difference

[1] Diet 6. Aqualim (closed formula)

Table 18. Amino acid profile of diets containing high inclusion of meat and bone meals (% of the diet).

	FM 100%	MBC 40%	MBC 60%	MBC 80%	MBC 100%	COMERCIAL
Aspartic acid	4.50	4.08	3.90	3.86	3.60	3.61
Threonine	2.10	1.88	1.77	1.73	1.65	1.72
Serine	2.53	2.28	2.18	2.18	2.08	1.81
Glutamic acid	6.51	6.28	6.03	6.17	5.90	5.72
Glycine	5.06	5.48	5.79	6.40	6.49	3.16
Alanine	3.35	3.34	3.39	3.65	3.51	2.70
Cysteine	0.41	0.45	0.45	0.50	0.54	0.44
Valine	2.29	2.17	2.12	2.12	2.07	2.26
Methionine	1.19	1.12	1.35	1.54	1.12	1.19
Isoleucine	2.04	1.77	1.63	1.56	1.43	1.65
Leucine	3.30	3.11	2.97	2.97	2.87	3.14
Tyrosine	1.32	1.14	0.98	1.08	0.96	1.08
Phenylalanine	1.81	1.69	1.62	1.55	1.46	1.67
Histidine	1.02	0.92	0.87	0.84	0.80	1.15
Lysine	3.40	2.99	2.73	2.53	2.28	2.78
Arginine	3.64	3.76	3.74	3.72	3.71	2.46
Tryptophan	0.39	0.35	0.33	0.30	0.27	0.38

3.3.3. RESULTS AND DISCUSSION

Average body weights of the gilthead seabream fed the test diets are shown in Figure 11. All fish accepted their respective experimental diets and fed aggressively during the experimental period. Table 19 summarizes the growth performance and feed utilization data obtained in the study.

The average body weights of the fish fed 60%, 80% and 100% MBC were significantly lower ($p<0.05$) than that of the control and 40% MBC fed groups. Feed efficiency was reduced from 60% for the control mixtures down to 22% for the 100% MBC diet. Protein efficiency ratio (PER), Specific growth rate (SGR) and apparent net protein utilization (NPU) all confirmed the findings. SGR ranged from 1.12 for the control group to 0.33 for the 100% MBC treated fish. Also PER declined from 1.38 for the fish fed the commercial ration to 0.49 for the 100% MBC group. Finally apparent NPU varied from 19.86 to 6.5 for the experimental conditions.

The carcass moisture, crude protein and ash was not found to be different among the fish fed the experimental diets. The lipid content of the fish, however, showed a significant decrease at higher MBC replacement levels. Amino acid profiles (Table 18) showed a decrease of lysine, tryptophan and methionine with increasing inclusion of meat and bone and this must be taken into consideration as limiting factors.

The subsequent histological examination showed no

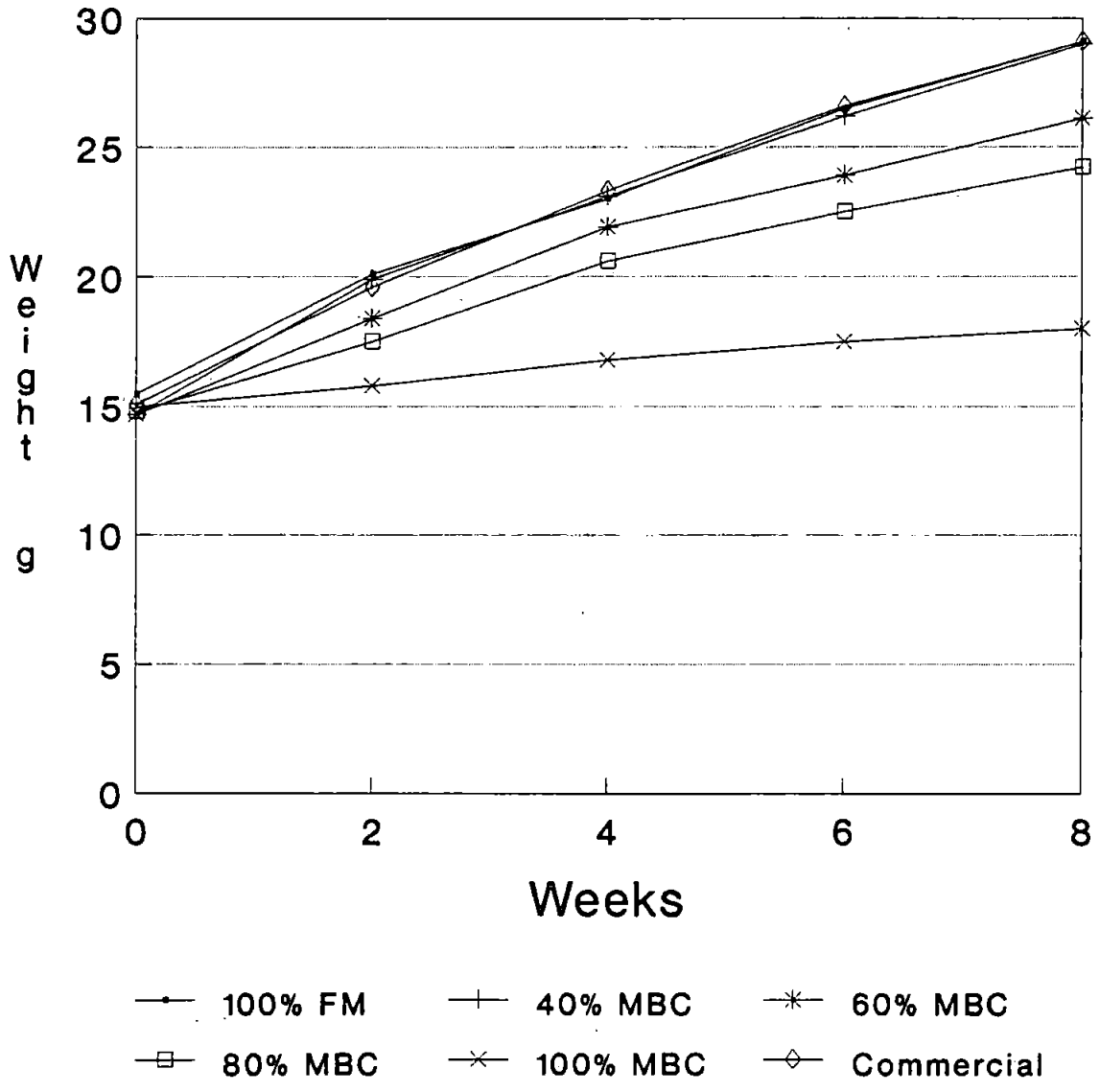

Figure 11. Mean weight increase of sea bream fed diets containing high inclusion of meat and bone meals.

Table 19. Growth performance and feed utilization of seabream fed experimental diets containing high levels of meat and bone meals.

	DIETS						
	FM 100%	MBC40%	MBC60%	MBC80%	MBC100%	COMERC	+SEM[1]
Initial mean weight (g)	15.54[a]	14.71[a]	14.60[a]	14.79[a]	15.97[a]	15.10[a]	0.35
Final mean weight (g)	29.15[a]	29.01[a]	26.14[b]	24.15[b]	18.00[c]	29.10[a]	0.82
Weight gain (%)	87.58	97.21	79.00	63.28	15.71	92.71	
Specific growth rate (%/day)	1.12	1.21	1.04	0.87	0.33	1.17	
Food intake (mg/day)	407.00	471.00	395.00	362.00	247.00	406.00	
Weight gain (mg/day)	243.00	255.00	206.00	167.00	54.00	248.00	
Feed efficiency (%)	59.52	54.14	51.28	46.08	22.47	61.35	
Protein efficiency ratio	1.28	1.22	1.18	1.03	0.49	1.38	
Nitrogen intake (mg/day)	30.40	33.50	27.50	25.90	17.00	29.10	
Nitrogen deposition (mg/day)	37.01	36.00	36.24	36.25	40.89	37.97	
Apparent net protein utilization	18.32	18.27	17.45	16.25	6.50	19.86	
CARCASS ANALYSIS (% wet weight)							
	Initial Fish						
Moisture	66.23	66.39[a]	66.65[a]	67.94[a]	68.51[a]	67.85[a]	66.98[a]	0.82
Protein	16.96	15.73[a]	15.98[a]	16.25[a]	16.49[a]	16.27[a]	15.76[a]	0.59
Lipid	10.78	12.00[a]	11.50[ab]	10.20[ab]	9.82[b]	9.95[b]	12.34[a]	0.65
Ash	3.72	3.64[a]	3.78[a]	3.89[a]	4.10[a]	4.24[a]	3.64[a]	0.12

Figures with common superscripts in each horizontal row are not significantly different ($p<0.05$)

pathological symptoms with respect to the morphology of the various tissues even at the highest inclusion levels.

Inclusion of meat and bone meal at levels of up to 40% of the total dietary protein did not seem to have a negative effect on growth and feed utilization of seabream fingerlings.

However, the growth performance of this fish was impaired at meat and bone inclusion levels contributing more than 40% of the dietary protein component. Probable reasons attributing to these findings are the high ash content of the product, its deficiency of certain amino acids as well as a suspected unsuitable fatty acid profile content.

It can be concluded that 40-50% of the protein is the maximum inclusion level of the meat and bone meals, used in this study, that seabream can utilize with no adverse effect on growth performance and feeding utilization.

3.4. EXPERIMENT 4

STUDIES TO ASSESS VARYING LEVELS AND SOURCES OF POULTRY DERIVED MATERIALS IN EXPERIMENTAL DIETS FOR SEABREAM

3.4.1. ABSTRACT

A 72 day trial was conducted using gilthead seabream (Sparus aurata) with an initial mean weight of 1.1g. The fish were fed with experimental diets containing various levels of differently treated poultry meals. A high fat poultry product replaced 20% of the fishmeal protein (PM 20%). The same product was hexane fat extracted and forced into the formulation at a higher level replacing 35% of protein (DPM 35%). Also, a high quality poultry meat meal was tested at three substitution levels of 20, 35 and 50% of the protein (PMM20%, PMM35%, PMM50%). The diets were formulated to be isonitrogenous and isocalorific to meet previously defined specifications.

All the diets performed better than the control fishmeal diet. Feed Efficiencies ranged from 63.3% to 53.5%, protein efficiency ratios (PER) varied from 1.08 to 1.24 and apparent net protein utilization from 19.19 to 21.29 for the tested diets. All these parameters supported the findings, suggesting that poultry meal is an excellent raw material which at levels up to 50% supports growth rates higher than fishmeal.

3.4.2 MATERIALS AND METHODS

3.4.2.1. Experimental fish and feeding regime

At the Polytechnic South West holding facilities, seabream fry, having an average initial weight of 1.1g were divided into six groups of 15. The fish were again supplied by the Cefalonia hatchery in Greece and fed six experimental diets over an eight week period. The fish were treated similarly to those used in the previous two experiments, the only difference being the feeding level which, for this size of fish, was set at 5% of their body weight daily.

3.4.2.2. Experimental facilities

The trial was conducted in the same experimental unit as described in section 3.3.2.2. and all the environmental conditions were monitored at the same levels as for the last two experiments.

3.4.2.3. Experimental diets

The formulations and proximate analysis of test diets and raw materials and the amino acid profile of the mixtures are summarized in Tables 20, 12, and 21 respectively.

The mixtures were formulated by substituting :

Table 20. Formulation of experimental diets for seabream using various poultry byproduct meals.

	DIET 1 FM 100%	DIET 2 PM 20%	DIET 3 DPM 35%	DIET 4 PMM 20%	DIET 5 PMM 35%	DIET 6 PMM 50%
White fish meal	74.01	59.19	48.10	59.19	48.10	37.00
Poultry meat meal(PMM)	-	-	-	15.78	27.62	39.45
Poultry meal (PM)	-	18.26	-	-	-	-
Poultry meal (DPM)	-	-	25.18	-	-	-
Cod liver oil	5.60	1.38	5.77	4.76	4.12	3.48
Starch/Dextrin	12.00	12.00	12.00	12.00	12.00	12.00
Ascorbic acid	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.40	0.40	0.40	0.40	0.40	0.40
Mineral premix	0.70	0.70	0.70	0.70	0.70	0.70
α-cellulose	6.20	6.97	6.75	6.06	5.96	5.87
Binder (guar gum)	0.50	0.50	0.50	0.50	0.50	0.50
NUTRIENT ANALYSIS (%)						
Moisture	4.81	5.03	6.03	5.46	5.14	7.74
Protein	47.11	47.22	48.30	48.75	48.61	47.99
Lipid	9.32	9.67	9.18	9.73	9.67	9.14
Ash	16.41	15.72	12.81	15.88	15.93	15.26
Fibre	8.09	8.18	7.98	8.43	8.40	7.87
NFE	14.26	14.18	15.70	11.75	12.25	12.00
Energy (MJ/Kg)	18.21	18.44	18.70	17.70	17.64	16.97

NFE calculated by difference

Table 21. Amino acid profile of diets containing different poultry byproduct meals for seabream (% of diet).

	FM 100%	PM 20%	DPM 35%	PMM 20%	PMM 35%	PMM 50%
Aspartic acid	4.11	3.99	4.09	4.10	4.07	4.04
Threonine	1.98	1.99	2.01	1.92	1.96	1.90
Serine	2.32	2.66	3.01	2.32	2.17	2.23
Glutamic acid	6.01	5.33	5.97	6.00	5.97	6.08
Glycine	4.08	4.04	4.07	4.14	4.26	4.30
Alanine	2.78	2.72	2.72	2.69	2.82	2.87
Cysteine	0.35	0.55	0.74	0.39	0.37	0.39
Valine	2.08	2.24	2.43	2.05	2.07	2.03
Methionine	1.40	1.20	1.12	1.30	1.27	1.17
Isoleucine	1.77	1.86	2.03	1.76	1.78	1.74
Leucine	3.05	3.19	3.36	3.09	3.07	3.10
Tyrosine	0.50	1.17	1.15	1.11	1.07	1.06
Phenylalanine	1.64	1.77	1.89	1.66	1.68	1.65
Histidine	0.50	0.97	0.97	0.93	0.92	0.91
Lysine	3.20	3.00	2.84	3.13	3.03	2.97
Arginine	3.17	3.31	3.41	3.20	3.05	3.78
Tryptophan	0.38	0.35	0.33	0.36	0.36	0.37

a. 20% of the fishmeal protein component with a standard high fat poultry meal (PM),
b. 35% of the protein by the same product after hexane extraction to reduce the crude lipid levels (DPM) and
c. 20, 35 and 50% of the protein by a high quality poultry meat meal (PMM) produced exclusively from poultry byproducts. This material has a good balance of amino acids and high digestibility and is processed by heating at 116^{o} C for four hours.

An all fishmeal (control) diet was also employed and each of the diets were formulated to be isonitrogenous (50% protein) and isocalorific (18 MJ/Kg G.E.). Crude fat and carbohydrate level was again adjusted at 10 and 12% of the diet respectively using cod liver oil and corn starch and dextrin. The diets were supplemented with minerals and vitamins according to Table 15 and prepared as described previously (section 2.3.4.).

3.4.2.4. Analytical techniques

The same chemical and statistical methods were used as described previously (section 3.2.2.4.).

3.4.3. RESULTS

Growth parameters, together with carcass analysis of the fish are presented in Table 22. It can be seen that all fish treated with the tested materials grew significantly better than those fed the control diet ($p<0.05$).

The formulations containing PM (20%) and DPM (35%) showed statistically the same results with respect to the final mean weights ($p<0.05$), but were significantly lower than the PMM (20%), PMM (35%) and PMM (50%) groups of fish. Total weight gain (%) and specific growth rate agreed with the forementioned. Feed efficiencies ranged from 63.3% for the PMM (35%) diet to 53.5% for the control group. The protein efficiency ratio for PMM (35%) was the best value obtained, followed by the PMM (50%) and PMM (20%) inclusions. The control diet gave the lowest relative growth performance. Apparent net protein utilization data indicated that all poultry meat meal inclusions were efficiently utilized in terms of protein retention.

Carcass analysis showed significant differences ($p<0.05$) among the fish fed the test ingredients. PM (20%) and DPM (35%) resulted in higher water content in the seabream, whereas PMM (20%), PMM (35%), and PMM (50%) gave lower values. Protein content was higher for the control group, PMM (20%) and PMM (50%). It was observed that in all

Table 22. Growth performance and feed utilization of seabream fed experimental diets containing various poultry byproduct meals.

	DIETS						
	FM 100%	PM 20%	DPM35%	PMM20%	PMM35%	PMM50%	+SEM[1]
Initial mean weight (g)	1.08[a]	1.11[a]	1.09[a]	1.11[a]	1.08[a]	1.11[a]	0.09
Final mean wight (g)	6.37	6.67[a]	6.53[a]	7.75[b]	7.50[b]	7.71[b]	0.69
Weight gain (%)	489.81	500.90	499.08	598.20	594.44	594.59	
Specific growth rate (%/day)	3.17	3.20	3.20	3.47	3.46	3.46	
Food intake (mg/day)	224.05	217.96	219.99	259.65	235.91	251.96	
Weight gain (mg/day)	119.52	129.05	125.48	148.10	149.76	151.67	
Feed efficiency (%)	53.34	59.17	57.14	57.14	63.29	60.24	
Protein efficiency ratio	1.08	1.19	1.11	1.11	1.24	1.16	
Nitrogen intake (mg/day)	105.55	102.92	106.26	126.58	114.68	120.92	
Nitrogen deposition (mg/day)	20.26	20.52	20.43	25.24	24.41	25.34	
Apparent net protein utilization	19.19	19.94	19.23	19.94	21.29	20.26	
CARCASS ANALYSIS (% wet weight)							
	Initial Fish						
Moisture	68.77	67.47[a]	68.20[b]	68.50[b]	66.07[c]	66.80[ac]	66.45[c] 0.58
Protein	15.33	16.95[a]	15.90[b]	16.28[bc]	17.04[a]	16.30[bc]	16.71[ac] 0.34
Lipid	8.71	8.15[a]	9.07[b]	8.56[a]	9.45[b]	9.24[b]	8.27[a] 0.32
Ash	4.31	4.61[a]	4.28[a]	4.12[a]	4.38[a]	4.21[a]	3.84[a] 0.27

Figures with common superscripts in each horizontal row are not significantly different ($p<0.05$).

[1]SEM Standard Error of the Mean calculated from the residual mean square in the analysis of variance.

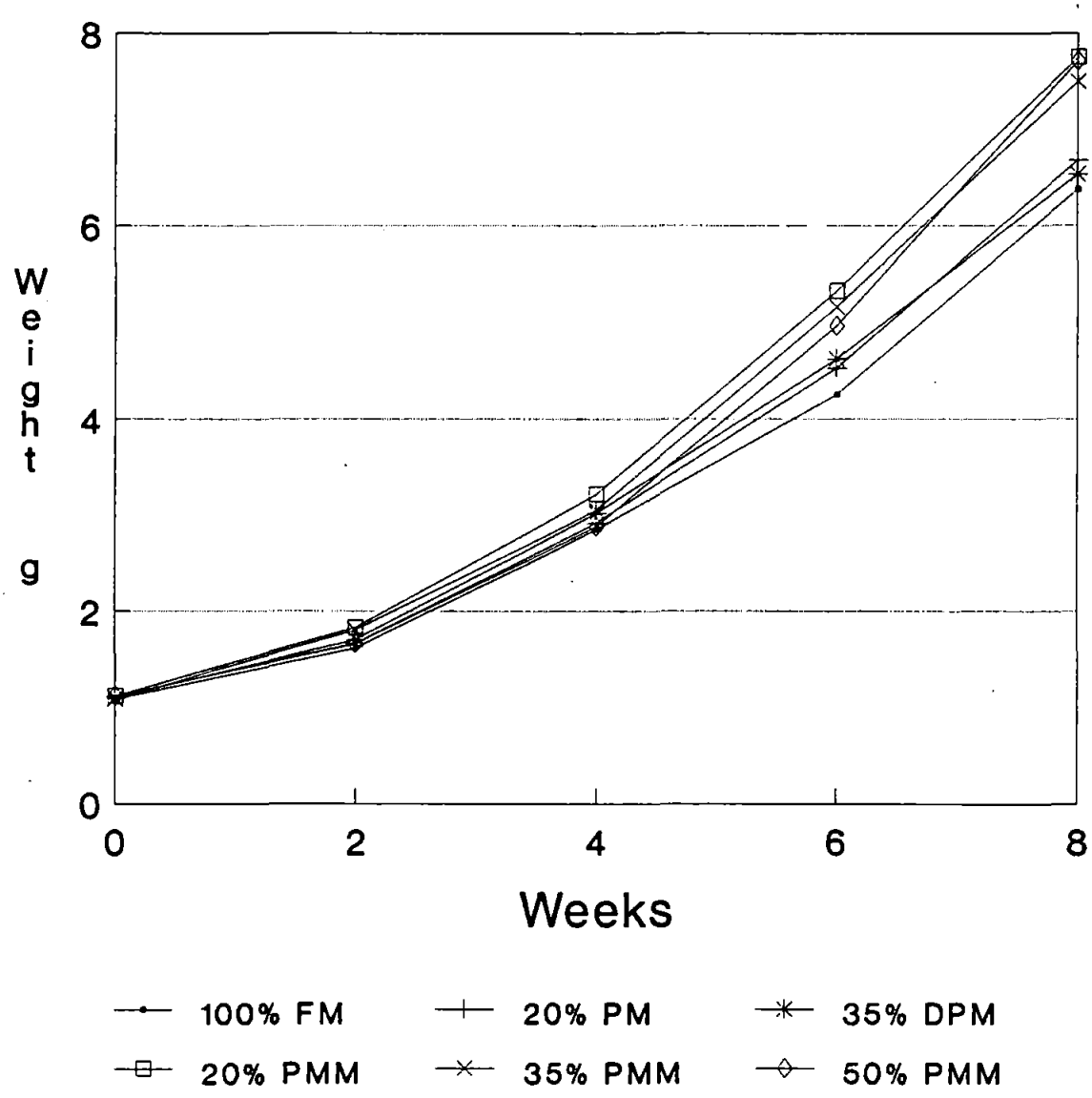

Figure 12. Mean weight increase of sea bream fed diets containing various Poultry byproduct meals.

treatments, the percentages of protein in fish at the end of the trial were higher than that found initially and this was in accordance with the previous experiments.

Gross body lipid contents of fish fed the PM (20%), PMM (20%) and PMM (35%) diets gave values which were significantly higher ($p<0.05$) than the remaining dietary treatments.

Substitution of poultry byproduct meals at levels as high as 50% of the total dietary protein resulted in better performance of seabream compared to those receiving the fishmeal control diet.

PMM diets gave slightly better results than PM and DPM mixtures and this can be attributed to the better amino acid profiles and especially the higher "available" lysine value.

Encouraging results from this study and also the meat and bone experiments suggested that raw materials of animal origin, having high protein contents and low carbohydrate levels, can be utilized by the carnivorous gilthead bream very efficiently. Further inclusion levels of poultry byproduct meals were therefore investigated in diets for the gilthead seabream to determine the limitations and constraints for this ingredient.

3.5. EXPERIMENT 5

FURTHER INCLUSION LEVELS OF POULTRY MEALS AND RELATED BYPRODUCTS IN DIETS FOR SEABREAM

3.5.1. ABSTRACT

In the previous experiment (section 3.4.) various poultry meals were successfully used to partially replace fishmeal at levels of up to 50% of the dietary protein. In this trial, the feasibility of further replacing fishmeal protein at levels of 75 and 100% with poultry meat meal was assessed.

Also, feather meal was tested at three substitution levels of 35, 50 and 75% of the protein component as well as a combined mixture of poultry meat meal and feather was tested at high inclusion levels of 75 and 100%.

Finally, a poultry byproduct meal which is produced for the Greek industry was tested at a level of 40% for comparison.

The diets were isocaloric containing 18MJ/Kg of gross energy, isonitrogenous (CP:45%) and had a lipid content of 12% on as fed basis. The experiment was carried out in a semiclosed rearing system and its duration was 84 days. The mean initial weight of the fish was 1.5g and the feeding level set at 4% of the body weight per day.

The groups of fish fed 75 and 100% poultry meat meal showed a slight reduction in all growth parameters compared to the control white fishmeal based formulation which, however, was not statistically significant. Similar results were obtained for the fish fed the poultry and feather mixture.

Feather meal alone at substitution levels of up to 50% produced no significant reduction in growth of seabream ($p<0.05$). At the level of 75% of the protein, however, the material caused a severe decrease in growth performance, feed efficiency, protein efficiency ratio and apparent net protein utilization. Inferior quality was also demonstrated by the poultry meal available on the Greek market.

3.5.2. MATERIALS AND METHODS

3.5.2.1. Experimental fish and feeding regime

Gilthead sea bream of mean weight 1.55g were obtained from Cefalonia Fisheries in Greece. After arrival, the fish were treated with oxytetracycline and left to acclimate for four weeks as described previously. The fish were then randomly distributed among eighteen rearing tanks at a rate of 17 fish per tank. The nine experimental diets were fed to duplicate groups of fish. The remaining stock were sacrificed and analyzed for gross carcass analysis.

All fish were fed a fixed ration of 4% of body weight per day in four separate meals for seven days per week. The fish were weighed at biweekly intervals and the feeding level adjusted accordingly. On the final day of the experiment all fish were sacrificed for subsequent chemical analysis and histological examination.

3.5.2.2. Experimental facilities

The experimental trial was conducted in a semi closed recirculation seawater system, situated at the fish nutrition

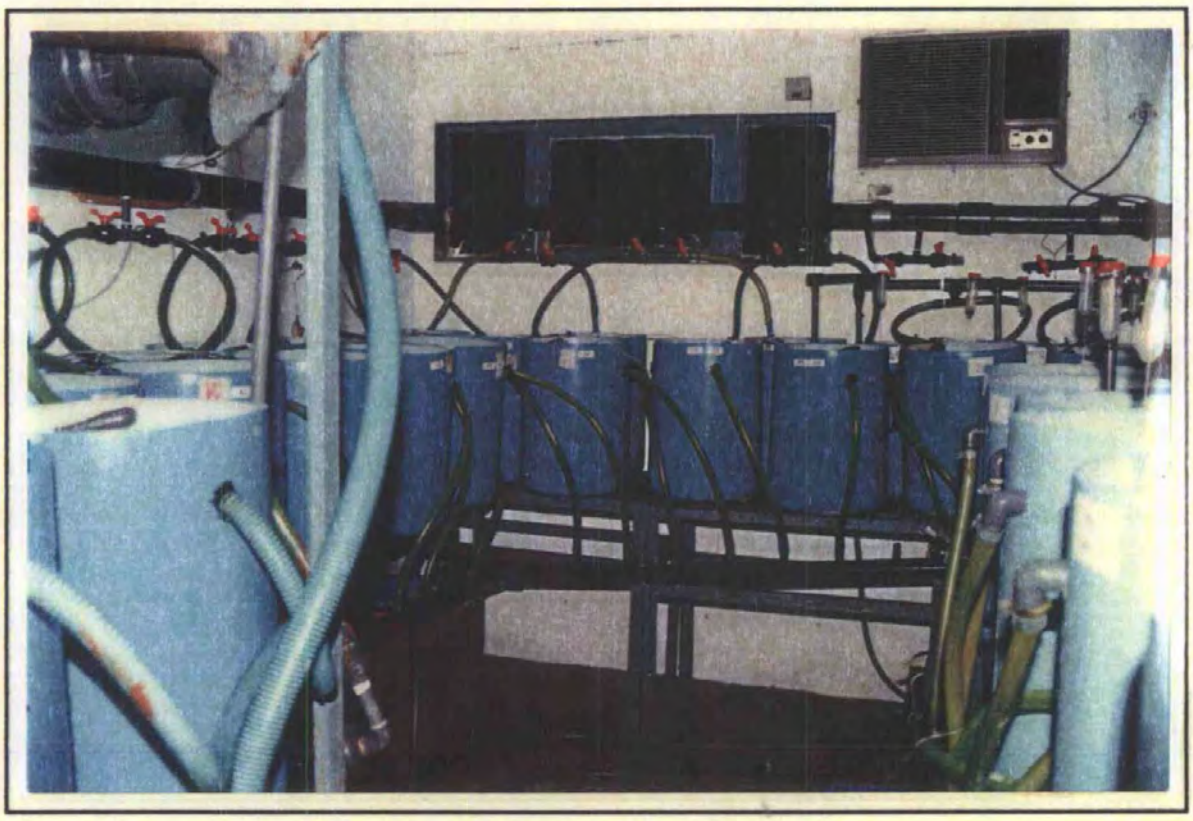

Plate 6. Facilities at NCMR (Greece) for experimental trials with marine fish.

aquarium of the National Center for Marine Research (NCMR) in Greece (Plate 6).

The system comprised of cylindrical tanks of 30l capacity connected on line with a 250l settling tank, a filtration unit and a 500l reservoir head tank where the temperature was controlled at 22^{o} C. The flow rate was adjusted to 1.5 l/min and the system was continuously supplied with fresh water at a rate of 1l/min to ensure a complete exchange of the water volume daily. All environmental conditions were held constant and maintained at the same levels as for all the previous experiments.

3.5.2.3. Experimental diets

The diet formulations and proximate analysis of the experimental mixtures are summarized in table 23. Amino acid profiles of the diets are shown in table 24.

The protein components used in the present investigation as replacements for fishmeal were:

a. The same poultry meat meal (PMM) which successfully replaced 50% of the fishmeal protein component in the previous study. PMM was included at two high substitution levels of 75 and 100% of the protein in the diets.

b. Hydrolyzed feather meal (FeM) at three inclusion levels of 35, 50 and 75% of the protein.

Table 23. Formulation of experimental diets for seabream using high inclusion levels of poultry byproduct meals.

	DIET 1 FM100%	DIET 2 PMM75%	DIET 3 PMM100%	DIET 4 Fe/P75%	DIET 5 Fe/P100%	DIET 6 PM140%	DIET 7 FeM35%	DIET 8 FeM50%	DIET 9 Fe M75%
White fish meal	72.90	17.98	-	17.98	-	42.18	45.70	35.15	17.98
Poultry meat meal(PMM)	-	53.55	71.40	-	-	-	-	-	-
Feather/Poultry (Fe/P)	-	-	-	50.25	67.00	-	-	-	-
Feather meal (FeM)	-	-	-	-	-	-	20.44	29.20	43.80
Poultry meal (PM1)	-	-	-	-	-	29.52	-	-	-
Cod liver oil	6.97	5.12	4.50	5.97	5.64	1.12	5.75	5.58	5.28
Starch/Dextrin	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Ascorbic acid	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium Phosphate	-	2.20	2.94	3.64	4.87	2.50	2.50	2.50	2.50
α-cellulose	3.53	3.55	3.56	4.56	4.89	6.58	7.51	9.47	12.74
Binder (guar gum)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
NUTRIENT ANALYSIS (%)									
Moisture	6.21	5.68	5.68	5.67	4.80	6.65	6.23	5.99	6.07
Protein	44.49	44.70	44.94	44.72	44.51	44.96	45.63	46.23	44.88
Lipid	12.53	12.55	12.43	12.00	14.11	14.43	12.56	13.12	12.50
Ash	15.28	14.10	13.52	12.81	12.35	12.17	11.98	10.50	7.20
Fibre	5.51	5.11	4.87	5.23	6.44	7.62	8.14	10.27	12.19
NFE	16.06	17.86	18.56	19.58	17.79	14.17	15.46	13.89	17.16
Energy (MJ/Kg)	17.64	18.08	18.21	18.08	17.98	18.62	18.45	19.12	18.39

NFE calculated by difference

Table 24. Amino acid profile of diets containing high inclusion levels of poultry byproduct meals for seabream (% of diet).

	FM100%	PMM75%	PMM100%	Fe/P75%	Fe/P100%	PM1 40%	FeM35%	FeM50%	FeM75%
Methionine	1.53	0.95	0.83	0.83	0.71	1.00	0.87	0.74	0.53
Met+Cysteine	1.90	1.47	1.24	1.60	1.56	1.61	1.82	1.88	1.98
Lysine	3.37	2.82	3.04	2.35	2.10	2.57	2.18	1.82	1.22
Tryptophan	0.42	0.41	0.49	0.38	0.33	0.46	0.40	0.36	0.30
Arginine	3.32	2.93	3.45	2.78	2.70	2.91	2.72	2.62	2.46
Histidine	1.70	1.39	1.44	1.11	1.02	1.29	0.94	0.76	0.46
Threonine	2.08	1.70	1.73	1.69	1.62	1.70	1.76	1.73	1.68
Isoleucine	1.85	1.82	1.64	1.78	1.63	2.00	1.96	1.87	1.71
Leucine	2.89	3.00	3.30	3.33	3.37	3.20	3.65	3.86	4.21
Valine	2.20	2.19	1.87	2.28	2.26	2.15	2.42	2.47	2.56
Phenylalanine	1.70	1.72	1.77	1.70	1.59	1.71	1.82	1.76	1.66

c. A mixture of PMM and FeM at a ratio of 3/1 (FeM/PMM), at substitution levels of 75 and 100% of the protein component.
d. A high fat poultry meal (PM1) available on the Greek market was forced in the formulation at a level of 40%.

An all white fishmeal based formulation was again used as a standard reference diet.

All the diets were designed to be isonitrogenous with a crude protein content of 45%, isocalorific with gross energy content of 18 MJ/Kg and to contain 12% of crude lipid.

Vitamins, minerals and carbohydrate sources were supplemented as described for all previous formulations.

3.5.2.4. Analytical techniques

The same analytical methods were again used as described in the previous experiments (section 3.2.2.4.).

3.5.3. RESULTS

Average final body weights and feed utilization data are presented in Table 25. Increase of the mean weight of fish fed each diet over the experimental period is illustrated in Figures 13 and 14. Fish fed 75% PMM; 100% PMM; 75% FeM/PMM; 35% FeM and 50% FeM only exhibited a slight reduction of final mean body weight compared to the fish fed the control diet. This difference, however, was not statistically significant ($p<0.05$). The 100% FeM/PMM fed gilthead seabream gave mean body weight values significantly lower than the control group, but not significantly lower compared to the values obtained for 75% PMM, 100% PMM, 75% FeM/PMM, 35% FeM and 50% FeM. Both groups of fish maintained on the 40% PM1 and 75% FeM diets yielded very low values.

Percentage weight gain and specific growth rate (SGR) followed the same general trends. SGR ranged from 2.4 for the all fishmeal fed fish to 1.56 for the 75% FeM group. Feed efficiency was generally high for all the diets except from the low value of 43.4% calculated for the 75% FeM formulation. Protein efficiency ratio (PER) varied from 1.40 to 0.89 and apparent net protein utilization from 24.38 to 13.26.

An increase in carcass moisture level was observed as the inclusion of feathermeal increased in the diet. This was followed by a decrease of crude protein and lipid content of the fish. The seabream fed 40% PM1 also contained higher

Table 25. Growth performance and feed utilization of seabream fed experimental diets containing high inclusion levels of various poultry byproduct meals.

	DIETS									
	FM100%	PMM75%	PMM100%	Fe/P75%	Fe/P100%	PM1 40%	FeM35%	FeM50%	FeM75%	+SEM[1]
Initial mean weight (g)	1.61[a]	1.55[a]	1.56[a]	1.49[a]	1.48[a]	1.63[a]	1.57[a]	1.57[a]	1.53[a]	0.26
Final mean wight (g)	12.09[d]	10.97[cd]	10.61[cd]	11.18[cd]	9.67[bc]	7.94[b]	11.63[cd]	11.00[cd]	5.69[a]	0.70
Weight gain (%)	653.38	608.54	579.79	651.02	556.06	388.65	640.61	601.92	271.34	
Specific growth rate (%/day)	2.40[d]	2.33[cd]	2.28[cd]	2.40[cd]	2.24[c]	1.89[b]	2.38[cd]	2.32[cd]	1.56[a]	0.21
Food intake (mg/day)	187.37	177.06	173.53	176.21	154.26	141.67	174.19	171.06	113.90	
Weight gain (mg/day)	124.76	112.20	107.68	115.36	97.56	75.18	119.76	112.20	49.52	
Feed efficiency (%)	66.54[c]	63.21[c]	62.05[c]	65.46[c]	63.24[c]	53.08	68.54[c]	65.68[c]	43.40[a]	2.09
Protein efficiency ratio	1.40[c]	1.34[c]	1.30[c]	1.39[c]	1.35[c]	1.08[b]	1.40[c]	1.34[c]	0.89[a]	0.04
Nitrogen intake (mg/day)	88.87	83.57	82.74	83.04	72.38	69.70	85.48	83.87	55.30	
Nitrogen deposition (mg/day)	20.62	21.00	18.49	18.76	16.66	12.62	20.94	18.90	7.36	
Apparent net protein utilization	23.20[c]	23.98[c]	22.35[c]	22.52[c]	23.02[c]	18.11[b]	24.38[c]	22.52[c]	13.26[a]	0.99
CARCASS ANALYSIS (% wet weight)										
	Initial Fish									
Moisture	74.47	69.88[bc]	67.67[a]	69.41[ab]	70.84[bc]	68.80[ab]	71.88[cd]	69.27[ab]	70.74[bc]	73.01[d]	0.64
Protein	16.70	16.57[bc]	17.73[d]	17.09[bcd]	16.30[b]	17.02[bcd]	16.76[bc]	17.35[cd]	16.82[bcd]	15.33[a]	0.30
Lipid	2.72	9.31[de]	10.17[f]	8.82[cde]	8.54[bcd]	9.81[ef]	6.83[a]	8.65[cde]	7.80[abc]	7.36[ab]	0.37
Ash	5.53	4.64[a]	4.75[a]	4.66[a]	4.49[a]	4.72[a]	4.79[a]	4.70[a]	4.72[a]	4.92[a]	0.14

Figures with common superscripts in each horizontal row are not significantly different ($p<0.05$)

[1] SEM Standard Error of the Mean calculated from the residual mean square in the analysis of variance.

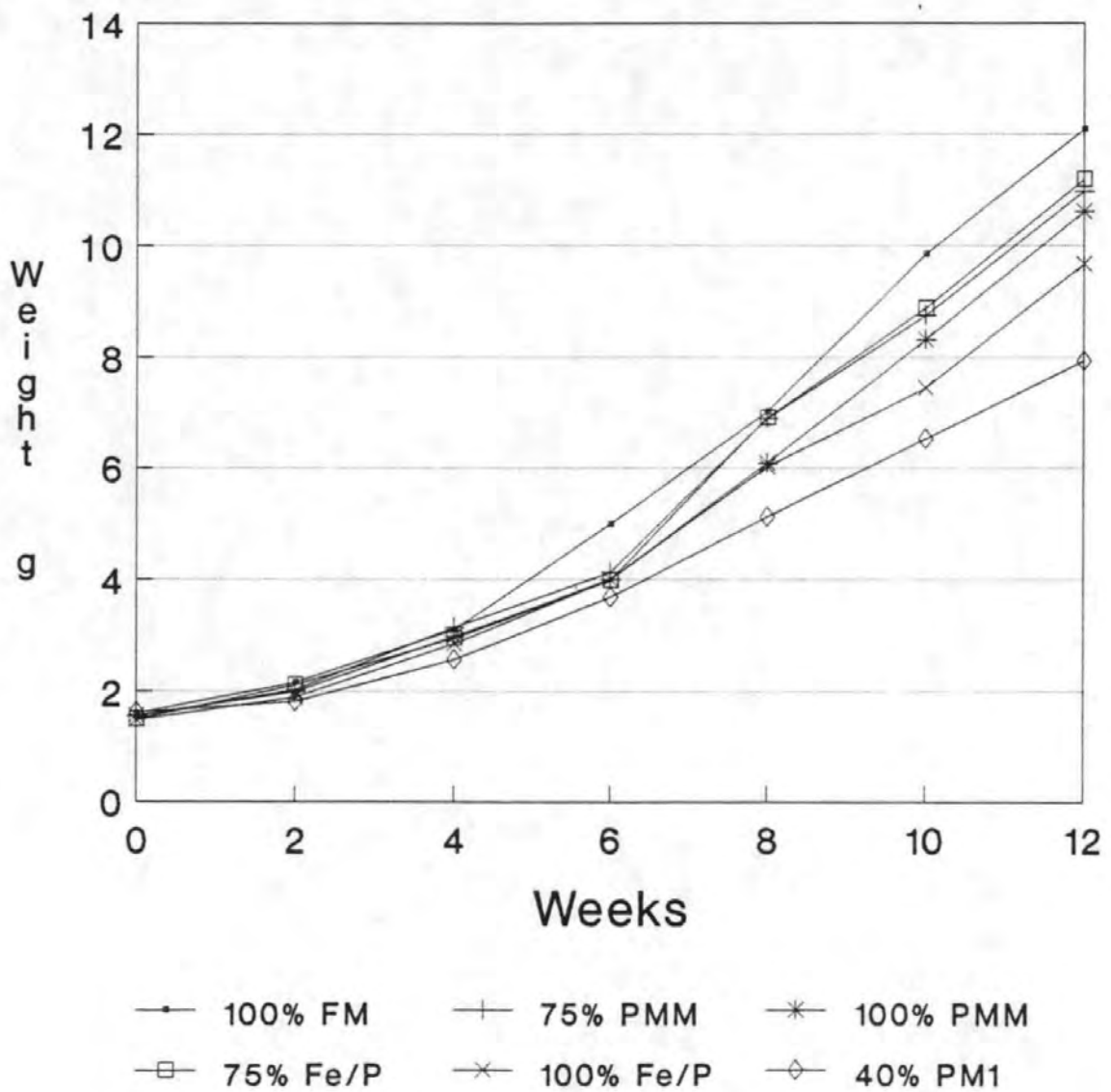

Figure 13. Mean weight increase of sea bream fed diets containing high inclusions of Poultry meals.

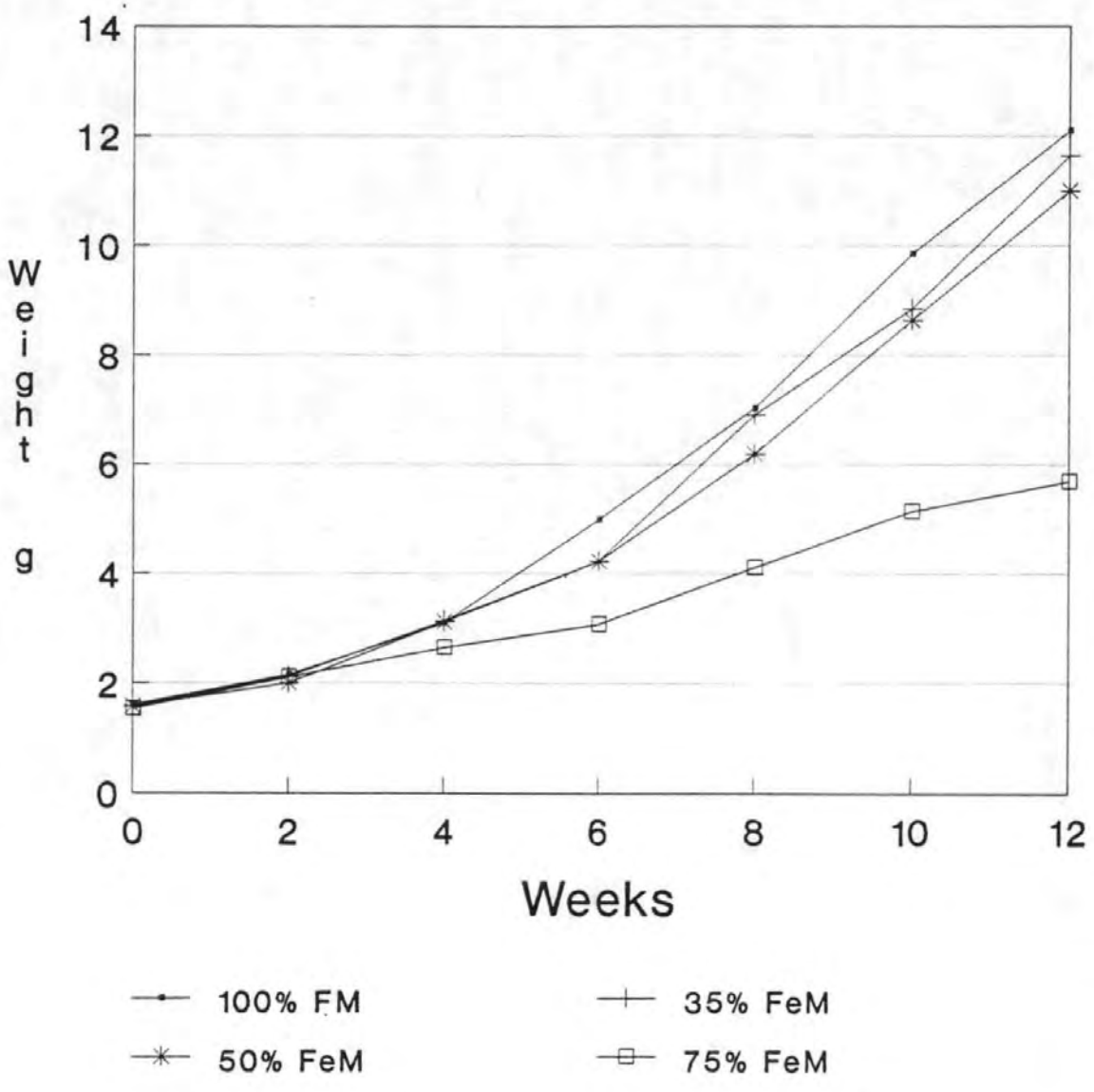

Figure 14. Mean weight increase of sea bream fed diets containing various inclusions of Feather meal

moisture and lower protein and lipid levels compared to the control group.

Despite the significant differences in growth, all fish remained in good health according to the histological examination undertaken on selected fish organs.

The seabream appeared to efficiently utilize poultry meat meal (PMM) at high or even complete inclusion levels. There was a slight decreased effect on growth but this was not statistically significant ($p<0.05$).

Poultry meat meal is a relatively good source of lysine and methionine. The content and availability of these essential amino acids, however, is lower than that of fishmeal (Allen, 1982). Lower "available" lysine values were determined for these products compared to fishmeal, especially for feather meal.

A probable unbalanced fatty acid profile in diets containing high inclusion levels of animal ingredients for seabream should also be considered.

The FeM/PMM mixture was also well utilized by seabream. Again amino acid deficiencies is one of the the most probable explanation for the decrease of fish performance.

At up to 50% FeM inclusion, the fish grew remarkably well. It seems that 50% fishmeal is enough to compensate for the unbalanced amino acid profile exhibited by feather meal.

High fat content and inferior processing procedures,

resulting in low protein quality and poor amino acid availability can be suggested as main reasons for the low performance of the fish fed the 40% PM1 diet.

3.6. DISCUSSION

Gilthead sea bream (Sparus aurata) seemed capable of utilizing most of the animal protein sources even at high levels of inclusion, with no adverse effect on the growth performance.

A variety of animal materials and byproducts have been tested for use in commercial feeds. Tacon et al. (1983a) showed that 50% of the fishmeal protein could be replaced by D,L-methionine supplemented meat and bone meal with no loss in survival, growth, feed efficiency and nutrient digestibility in tilapia (Oreochromis niloticus). In agreement with these findings Fishelson and Yaron (1983) found that 50% of brown fishmeal protein could be successfully replaced by hexane extracted meat and bone meal for the same species. Encouraging results were also obtained by Langar and Metailler (1989), who replaced 30% of the protein with meat and bone meal in seabass (Dissentrarchus labrax) diets with no impairment of growth and feed utilization. Finally Davies et al. (1989a) found that meat and bone meal can effectively replace up to 75% of fishmeal in practical diets for tilapia (O. mosambicus).

Conflicting results were obtained by Fowler and Banks (1976) on chinook salmon (Oncorhynchus tsawytscha). Fish fed formulations containing 10-30% meat and bone meal all

exhibited a lower growth rate and feed utilization compared to the control group.

In the present studies with meat and bone meal, seabream utilized this animal protein source efficiently at inclusion levels of up to 40% of the protein component. Relatively good nutrient digestibility measured for this product supported these findings. At higher inclusion levels, however, amino acid deficiency, lower lysine availability, fatty acid profile and high ash content are the likely factors adversely affecting growth performance.

Lipromel$_R$ was well utilized by seabream at the level tested. Similar results are reported for rainbow trout (Oncorhynchus mykiss) in which fish performed better than those fed the reference practical type diet when Lipromel$_R$ was included at a level of 22.5% in the diet (Commercial data, Prosper de Mulder ltd, technical report). Excess animal fat content within this product, however, makes further substitution a problem, especially for marine fish species such as seabream. High inclusion of animal fat is not recommended in diets for marine fish because of their reported inability to elongate and desaturate lower polyunsaturated fatty acids of the n-3 series to C20 or C22 highly unsaturated fatty acids compared to freshwater fish (Yone et al., 1974; Cowey, 1976; Millikin, 1982). Alexis (unpublished data) found that a depression of growth occurred when seabream fingerlings were fed diets containing marine

oil at levels lower than 6% in the diet. In contrast, salmonids have exhibited some degree of tolerance to high animal fat inclusions in their diet. Dosanjh et al. (1984) found pork lard to be an excellent source of lipid for coho salmon (O. kisutch). Similarly Mugrditchian at al. (1981) reported no significant differences in growth of chinook salmon fed beef suet as dietary fat in the diets. Temperature and the characteristic physiological status of the fish species under consideration, are obviously important criteria with respect to this latter point.

A number of investigations have been directed towards the evaluation of different poultry byproduct meals in diets for several farmed fish. Tiews et al. (1976) demonstrated that fishmeal can be entirely replaced without a reduction in feed efficiency in diets for rainbow trout by a mixture of poultry byproduct meal and feathermeal together with lysine, D,L-methionine and tryptophan supplementation. This finding agrees with the work of Gropp et al. (1979) where even a complete replacement of fishmeal by poultry byproducts, only resulted in a slight depression of growth parameters. A better performance of rainbow trout when the fishmeal content in their diets was partially substituted by poultry byproducts has also been reported by Alexis et al. (1985). Steffens (1985), also showed that poultry waste meal can be used as partial substitute (30%) for fishmeal in rainbow

trout feeds. In a later experiment Steffens (1987) found that an experimental diet containing 53% poultry by product meal as a sole animal protein source does not provide significant differences in growth and feed efficiency when compared to an isonitrogenous control fishmeal based diet. Pokorny (1982) also recommends the enrichment of feed mixtures for the rearing of rainbow trout with 20 to 40% poultry meal and 8 to 10% poultry fat. Higgs et al. (1979) reported that the quality of protein from defatted poultry byproduct meal alone or from a mixture with hydrolyzed feather meal approaches that of herring meal in diets fed to coho salmon (O. kisutch).

Feather meal is often considered to be an inferior source of protein for fish because of its poor digestibility and essential amino acid profile. In contrast, Koops et al. (1982) successfully formulated diets containing 14-15% feather meal for rainbow trout (Oncorhynchus mykiss); whilst Tiews et al. (1979) also successfully replaced 50% of the protein with feather meal in rainbow trout diets. Finally Fowler (1990) testing inclusion of up to 15% of the dietary protein with feather meal observed no adverse effect on the growth and feed utilization of chinook salmon.

All these forementioned findings with respect to the feasibility of using poultry derived products in formulations for various fish species, are in agreement with the results reported for this present investigation.

The potential for considering these animal protein sources for the gilthead seabream is therefore promising. In the search for alternative ingredients, attention was next directed towards plant based materials. Previous digestibility data and reference to ingredient availability indicated that soyabean derived products offered the best scope for research in relation to seabream nutrition.

CHAPTER 4

SUBSTITUTION OF FISHMEAL WITH SOYABEAN MEAL PRODUCTS AND DERIVATIVES

4.1. INTRODUCTION

The increasing cost and short supply of fish meal over the last decade has contributed to higher prices for marine fish feeds because of the high percentage of fish meal used in most commercial formulations. Hence, interest has been directed towards evaluating less expensive plant protein sources in complete diets for a number of fish species (Tacon and Jackson, 1985).

However, most of these protein sources contain substances that might cause slight to severe negative effects on the nutritional status of an animal. Insoluble fibers, soluble fibers, enzyme inhibitors, saponins, lectins, tannins, phytic acid and gossypol are the most important antinutrients acting in the gastro intestinal tract (Krogdahl, 1986).

Antinutrients affect digestive function and nutrient absorption by :

1. Altering the flow of chyme and secretions.
2. Impairing the interaction between nutrients and digestive components.

3. Restricting the diffusion of nutrients.
4. Altering the nature absorptive surfaces.
5. Changing microbial activity.

Dietary fibres

The insoluble fibres comprise cellulose, most hemicelluloses and lignin, which all pass the gastrointestinal tract without major alteration except a certain swelling.

The soluble fibres, however, comprising some hemicelluloses, pectins, gums, oligosaccharides and other minor components, may be altered during the intestinal passage and change characteristics on their way (Wallace and Bell, 1983). Oligosaccharides may comprise more than 10% of legume seeds (Saini, 1989). The extraction of oligosaccharides from soyabean, with methanol for example has been found to improve the utilization of nutrients by chum salmon (Oncorhyncus keta) and rainbow trout (Murai et al., 1987; Murai et al., 1989a).

The insoluble fibres appear to increase intestinal passage rate whereas the soluble decrease this rate (Meyer et al., 1988).

Increased rates tend to decrease nutrient absorption (Krogdahl, 1985). On the other hand, the decreased flow rate caused by soluble fibre is not always followed by increased absorption of nutrients. Other characteristics of soluble

fibers such as gel formation water binding, cation exchange and antioxidation, impair digestive processes (Eastwood and Kay, 1979). Moreover the slowing effect on flow rate may decrease feed intake with negative effects on production (Bishawi and McGinnis, 1984).

Fibrous components of plant cell walls may also act as insulators between the nutrients and the digestive medium. Additionally, soluble fibres have been shown to inhibit digestive enzymes in vitro (Jakson et al., 1982).

The strong ion exchange characteristics of some of the fibres may reduce the bioavailability of important dietary minerals, particularly the vitamins and minerals such as vitamins B_{12}, Ca, Mg, Zn, and Cu.

Evidence exists that dietary fibres decrease the available bile salts causing the liver and gallbladder to respond with increased secretion. The decrease in lipid absorption often seen, may partly be due to incomplete replacement of the bile salts drained from the organism by the fibre complex in digesta (Vahouny et al., 1988).

Dietary fibre is known to affect intestinal receptors, not only those regulating flow of chyme, secreta and feed intake, but also receptors mediating signals to the internal metabolism of nutrients (Anderson and Chen, 1979).

By slowing down the intestinal transit rate, soluble fibres change the environment of microflora and increase the time of exposure to substrates. The fibres themselves are

substrates for the microflora. They also carry along unabsorbed proteins, carbohydrates, lipids and minerals (Cummings and Englyst, 1987). The impact of the microflora may be interpreted from the fact that germ free animals may grow faster and utilize feed superior to the conventional situation and that antibiotics are used to improve feed utilization (Campell et al., 1983).

Enzyme Inhibitors

Specific inhibitors of digestive enzymes have been found in most of the common plant feedstuffs (Richardson, 1980; Gargouri et al., 1984). In the intestinal lumen the inhibitors prevent enzymes from taking part in the digestion of nutrients. Enzyme inhibitors may alter flow of digesta and secreta, enlarge pancreas and cause accumulations of undigested nutrients which feed the microorganisms (Mei, 1985). Digestive enzymes contain high concentrations of the amino acid cycteine. The increase in secretions of enzymes, triggered by the inhibitors has been reflected in a several fold increase in the conversion of methionine to cycteine. This would probably increase the requirements of sulphur amino acids in the diets of intensively fed animals, including fish (Richardson, 1980).

A major part of the effects appear to be due to a change in gut associated microbial activity. Decreased digestive activities increase nutrient supply to the microflora and

stimulates its growth (Niess, 1980).

Other Plant Components

Several other components in plant protein feedstuffs may affect protein adversely by interfering with digestive processes.

Phytic acid, often associated with the fibre fraction of plant material, possesses specific binding sites for divalent ions resulting in a negative effect on mineral availability. Zinc utilization was affected negatively by phytic acid in investigations with channel catfish (Ictalurus punctatus) (Satoh et al., 1989) and rainbow trout (Spinelli et al., 1979).

Saponins appear to disturb cholesterol metabolism. However very little is known about their mode of action. Cholesterol supplementation may overcome some of the retarding effects (Liener, 1980).

Tannins interfere with protein and dry matter digestibility by inhibiting the gastrointestinal proteases and possibly other enzymes by forming indigestible complexes with dietary proteins (Huisman et al., 1989).

Lectins, some of which are highly toxic, appear to cause general impairment of nutrient absorption. They show variable resistance to proteolysis and are characterized by the ability to bind carbohydrate containing membrane receptors. In addition, disturbances of the intestinal bacterial ecology

have also been reported (Huisman et al., 1989).

Several approaches of inactivation or removal of antinutrients have been pursued. These include heat treatment, irradiation, soaking and extraction with water or other fluids, fermentation, enzyme treatment of ingredients, or supplementation of diets with enzymes hydrolyzing specific antinutrients. Some of these may become increasingly important as cheaper biotechnological processes are developed (Lovell, 1980; Viola et al., 1983).

The use of full fat soyabean meal in fish feeds has received attention since research showed that heating full fat soybeans at a temperature of 177^{o} C or above, improved the nutritional value for trout above that achieved by commercial processing methods (Lovell, 1985).

The apparent nutritional benefits of properly heated, full fat soybean meals over commercial solvent extracted meal are improved protein quality, due to the fact that additional heating destroys more of the antinutritional factors, and additional energy because of the high oil content of this plant protein material. Also, the oil present in the processed beans, contains significant levels of lecithin which is a natural emulsifier. Thus it can be assumed that the digestibility of the oil will be enhanced over samples of commercial soya oil from which lecithins have been removed (Holmes, 1987). Another advantage of the full fat soya meal is that its oil is very stable, thus giving a remarkably long

shelf life for such a high fat product. This is because of the high levels of tocopherols present in the oil which act as natural antioxidants (Holmes, 1987).

The successful conversion of the soyabean into a high energy material for livestock feeding requires a carefully controlled process involving the correct amount of steam, temperature and residence time. Whilst the process may well be a continuous one, there is constant potential change in the system and strict quality control is required to ensure:

a. A maximum reduction of trypsin inhibitor levels in the product,

b. optimal cooking to ensure good protein and amino acid availability and;

c. uniform gross nutrient composition.

Various analytical methods are employed for the quality control procedure in full fat soya production for animal feeds. Trypsin inhibitor activity (TIA), the urease test, protein dispersibility index (PDI), cresol red and "available" lysine content are the most common analytical procedures for the quality control of this material.

It is evident that soyabean processing technology has led to a number of new products and derivatives in the feed industry. The variation in quality and nutritional value are important considerations for studies designed to test their feasibility in diets for seabream.

4.2. EXPERIMENT 6

PARTIAL SUBSTITUTION OF FISHMEAL WITH SOLVENT EXTRACTED SOYABEAN MEAL

4.2.1. ABSTRACT

A 72 day feeding trial was conducted using seabream fingerlings (Sparus aurata) with an initial mean weight of 6.2g. The fish were fed six diets containing different percentages of solvent extracted soybean meal as a replacement for white fishmeal at four substitution levels, 10, 20, 30 and 40% of the fishmeal protein component. A control diet without soybean meal inclusion and a commercial marine fish diet were also employed. The experimental diets were formulated to be isonitrogenous with 48% digestible crude protein and similar with respect to dietary lipid (10% crude lipid) and carbohydrate content.

In declining order the diets supported less growth as the inclusion of soybean meal increased. However, at the 20%-30% substitution level the growth was not significantly lower than the increase obtained for fish fed the control and the commercial diet ($p<0.05$). Feed efficiency ranged from 72.5% to 64.9% for the commercial feed and the 40% soy bean diet respectively. Also, fish fed diets containing soybean protein showed reductions, compared to the control group, in

protein efficiency ratio (PER), specific growth rate (SGR) and apparent net protein utilization (NPU). There were no significant differences between the proximate composition of the carcasses of the fish fed all the experimental diets.

4.2.2. MATERIALS AND METHODS

4.2.2.1. Experimental fish and feeding regime

Gilthead seabream (Sparus aurata) fingerlings having a mean weight of 6.21 g were obtained from Cefalonia hatchery in Greece. The stock was acclimated to the new environmental conditions for one month and then they were randomly distributed to the six experimental tanks for the 72 day feeding trial. The stocking density was decided to be forty fish per tank and the fish were treated as described in section 3.2.2.1., before, during and on termination of the experiment.

4.2.2.2. Experimental facilities

The experiment was conducted in a recirculating sea water system in Polytechnic South West as described in section 3.2.2.2. All water and environmental parameters were monitored and held at similar levels as with all the previous trials.

4.2.2.3. Experimental diets

The formulations and proximate analysis of the test diets are shown in Table 27. Amino acid profiles of the mixtures

Table 26. Nutrient composition of raw materials tested.

	(% as recieved)						
	Moisture	Protein	Lipid	Ash	Fibre	NFE	Available Lysine (% protein)
White fish meal	2.82	66.18	8.63	21.12	2.55	-	6.54
Soya bean meal (Solv.extr.)	10.86	43.05	1.80	6.00	9.23	29.06	6.46
Full fat soya meal (LS)	6.82	37.03	20.15	4.76	10.01	21.11	6.52
Full fat soya meal (SS)	14.38	34.48	19.31	4.51	11.31	17.42	6.51
Full fat soya meal (HS)	11.65	35.57	19.83	4.42	7.55	20.98	6.21
Danpro-A	6.51	66.00	0.25	4.66	8.32	14.26	5.56

NFE calculated by difference

Table 27. Formulation of experimental diets for seabream using solvent extracted soyabean meal.

	DIET 1 FM 100%	DIET 2 SM 10%	DIET 3 SM 20%	DIET 4 SM 30%	DIET 5 SM 40%	DIET 6 COMMERCIAL[1]
White fish meal	74.01	66.61	59.21	51.81	44.41	*
Soyabean meal	-	11.87	23.74	35.61	47.48	*
Cod liver oil	3.61	4.04	4.47	4.90	5.32	*
Starch/Dextrin	14.00	10.56	7.12	3.67	0.20	*
Ascorbic acid	0.10	0.10	0.10	0.10	0.10	*
Choline chloride	0.50	0.50	0.50	0.50	0.50	*
Vitamin premix	0.40	0.40	0.40	0.40	0.40	*
Mineral premix	0.70	0.70	0.70	0.70	0.70	*
Dicalcium phosphate	-	-	-	-	0.60	*
α-Cellulose	6.38	4.92	3.46	2.01	-	*
Binder (guar gum)	0.30	0.30	0.30	0.30	0.30	*
NUTRIENT ANALYSIS (%)						
Moisture	6.54	6.48	5.60	6.25	5.50	8.50
Protein	47.50	47.70	47.70	48.00	48.10	49.65
Lipid	9.56	9.45	9.62	9.72	10.00	11.90
Ash	16.15	15.39	14.72	14.50	13.95	10.76
Fibre	6.11	5.49	5.27	6.75	6.90	1.62
NFE	14.14	15.49	17.09	14.75	14.55	17.57
Energy (MJ/Kg)	17.63	17.83	18.36	18.29	18.56	19.44

NFE Nitrogen Free Extracts calculated by difference.
[1]Diet 6. Aqualim (closed formula).

Table 28: Amino acid profile of diets containing various inclusions of solvent extracted soyabean meal.

	FM 100%	SM 10%	SM 20%	SM 30%	SM 40%	Commercial
Aspartic acid	4.35	4.48	4.59	4.91	5.04	4.45
Threonine	2.08	2.02	2.04	2.03	2.11	2.19
Serine	2.51	2.52	2.47	2.55	2.71	2.26
Glutamic acid	6.39	6.49	6.92	7.22	7.63	7.06
Glycine	4.30	3.92	3.82	3.61	3.48	3.24
Alanine	2.95	2.74	2.73	2.66	2.63	3.00
Cysteine	0.37	0.44	0.46	0.49	0.51	0.48
Valine	2.20	2.74	2.26	2.25	2.31	2.66
Methionine	1.53	1.41	1.30	1.25	1.28	1.34
Isoleucine	1.85	1.87	1.98	2.02	2.07	1.91
Leucine	2.89	3.90	3.29	3.33	3.47	3.85
Tyrosine	1.19	0.81	1.89	1.26	1.47	1.09
Phenylalanine	1.70	1.71	1.40	1.95	2.08	2.06
Histidine	0.98	1.05	1.10	1.19	1.26	1.52
Lysine	3.37	3.32	3.34	3.30	3.36	3.66
Arginine	3.32	3.34	3.49	3.55	3.63	3.12
Tryptophan	0.42	0.43	0.46	0.48	0.51	0.52

are shown in Table 28, and the gross chemical composition of the ingredients used in Table 26.

The experimental diets were prepared by replacing four levels (10, 20, 30 and 40%) of the fishmeal protein component with a solvent extracted soyabean meal. A control diet with 100% white fishmeal and a commercial marine fish feed were also employed as controls. All the diets were designed to be isonitrogenous (50% crude protein) and isocalorific (18 MJ/Kg gross energy). The crude lipid was adjusted at a level of 10% of the diet by supplementation with cod liver oil. Addition of mineral and vitamin premixes were included in all treatments and a corn starch / dextrin 2:1 mixture was used to adjust the carbohydrate level to 14% for all the formulations. Dicalcium phosphate was also added to the diet containing 40% soybean meal to compensate for any calcium and phosphorus deficiencies resulting from lowered levels of fishmeal.

4.2.2.4 Analytical techniques

The same chemical procedures were employed in this investigation as described previously (section 3.2.2.4).

4.2.3. RESULTS

All fish soon accepted the experimental diets and fed aggressively for the duration of the experiment. The growth performance and feed utilization values and carcass analysis of the fish are displayed in Table 29. The results show that on the basis of mean final body weight there was no significant difference between the control diet, the commercial diet and the two diets replacing 10 and 20% of the fishmeal protein ($p<0.05$). A further increase of soybean inclusion, however, resulted in a statistically significant growth depression ($p<0.05$). Specific growth rate (SGR) values and percentage weight gain followed the same trend. Feed efficiency was not noticeably different for all diets except the one with the higher soybean inclusion level. Protein efficiency ratio (PER) and apparent net protein utilization (NPU) resulted in similar values for all of the five diets but the 40% soyabean meal formulation produced lower values .

There were no significant differences ($p < 0.05$) in the body composition of the fish fed all the experimental diets, although fish fed the diets containing 30 and 40% soybean meal gave slightly higher overall protein contents. Also seabream fed on the commercial diet had a small decrease in water content and an increase of whole body lipids.

Histological examinations showed no changes with respect

Table 29. Growth performance and feed utilization of seabream fed experimental diets containing varying levels of solvent extracted soyabean meal.

	DIETS						
	FM 100%	SM 10%	SM20%	SM30%	SM40%	Commercial	+SEM[1]
Initial mean weight (g)	6.27[a]	6.13[a]	6.17[a]	6.21[a]	6.20[a]	6.27[a]	0.12
Final mean weight (g)	29.03[a]	27.56[ab]	28.85[ab]	26.40[bc]	24.91[c]	30.67[a]	0.71
Weight gain (%)	362.00	349.59	367.59	325.12	301.77	389.15	
Specific growth rate (%/day)	1.82	1.79	1.83	1.72	1.66	1.89	
Food intake (mg/day)	455.20	440.51	447.30	415.02	400.18	467.67	
Weight gain (mg/day)	316.11	297.64	315.00	280.42	259.86	338.89	
Feed efficiency	69.44	67.56	70.42	67.57	64.93	72.46	
Protein efficiency ratio	1.37	1.31	1.39	1.31	1.25	1.34	
Nitrogen intake (mg/day)	216.22	210.12	213.36	199.21	196.49	232.20	
Nitrogen deposition (mg/day)	53.61	49.65	53.01	48.29	44.80	57.48	
Apparent net protein utilization	24.79	23.63	24.84	24.24	22.80	24.75	
CARCASS ANALYSIS (% wet weight)							
	Initial Fish						
Moisture	68.99	67.19[a]	67.06[a]	67.97[a]	67.06[a]	67.25[a]	66.23[a]	0.35
Protein	15.12	16.96[a]	16.68[a]	16.83[a]	17.22[a]	17.24[a]	16.96[a]	0.21
Lipid	9.31	9.98[a]	9.75[a]	9.16[a]	9.74[a]	9.59[a]	10.82[a]	0.18
Ash	3.98	3.81[a]	3.82[a]	3.91[a]	3.70[a]	4.00[a]	3.74[a]	0.11

Figures with common superscripts in each horizontal row are not significantly different ($p < 0.05$).

[1]SEM Standard Error of the Mean calculated from the residual mean square in the analysis of variance.

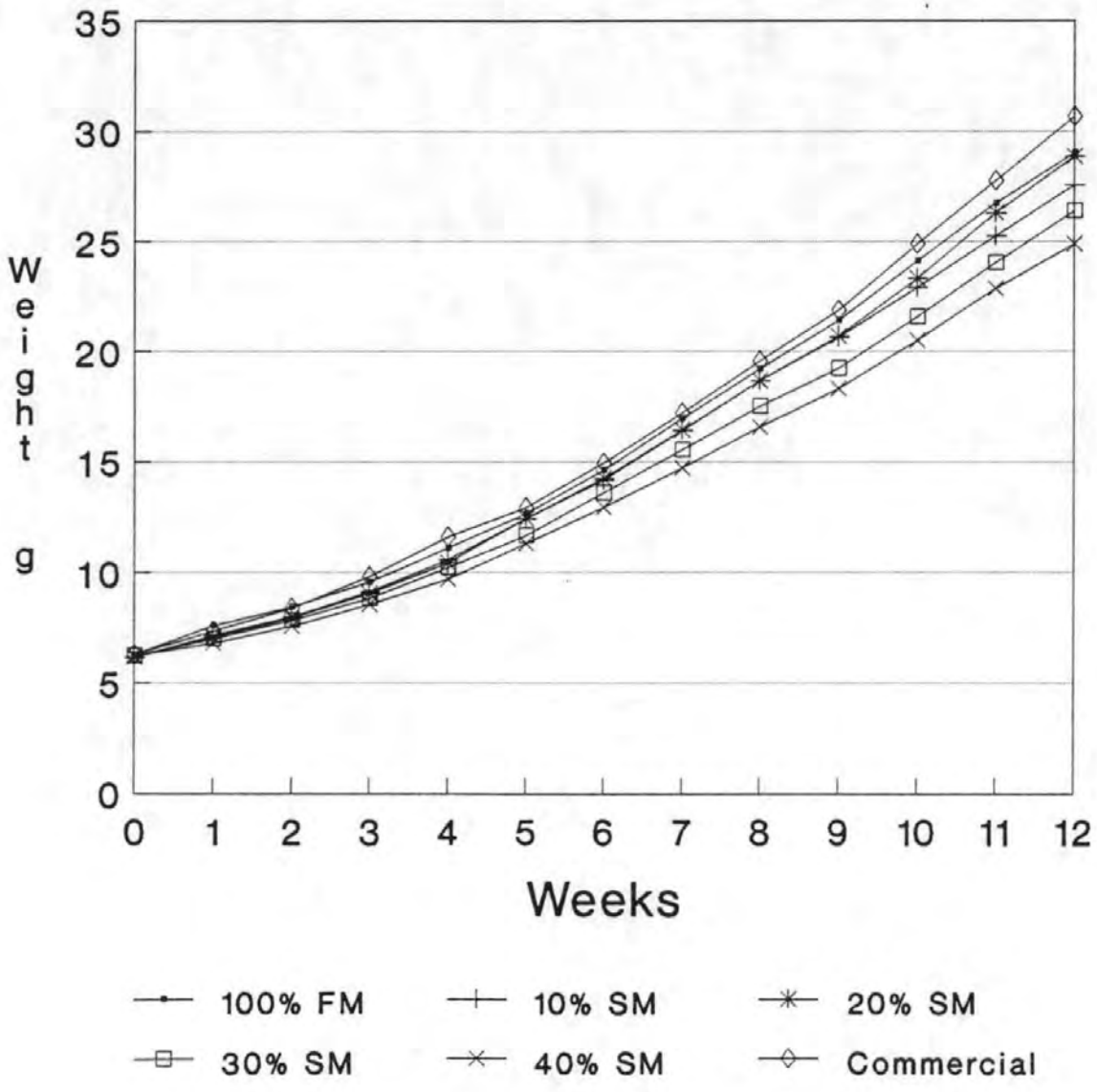

Figure 15. Mean weight increase of sea bream fed diets containing various inclusions of soyabean meal.

to the morphology of tissues from selected organs.

It is concluded from the present study that the standard solvent extracted soyabean meal used in the present study can only be used to substitute not more than 20-30% of the diet for the gilthead seabream, with no impairement of the growth performance and feed utilization.

As a next step, the effect of different types of processing technology on the nutritional value of soyabean meals in diets for seabream were investigated.

4.3. EXPERIMENT 7

PARTIAL SUBSTITUTION OF FISHMEAL WITH DIFFERENTLY PROCESSED SOYABEAN MEALS AND SPECIALIZED SOY PRODUCTS

4.3.1. ABSTRACT

Six isonitrogenous and isocaloric diets, with 35% of the total protein contributed from differently processed soyabean meals, were fed to juvenile Sparus aurata. The fish (average weight 1.6g) were fed for 84 days at a constant feeding level of 4% of the body weight per day. All diets were formulated to contain 45% crude protein, 12% crude lipid and 18 MJ/Kg gross energy.

The soyabean meal supplements used were three full fat meals heated for various time lengths, a solvent extracted meal and a soya protein concentrate. A control formulation consisting of white fishmeal was also employed. Protein digestibility coefficients were measured for all the experimental diets.

Available lysine, trypsin inhibition activity and cresol red determination were undertaken to assess the quality characteristics of the meals.

The final mean weights, specific growth rate, and feed

efficiency of the fish fed the underheated full fat meal, solvent extracted meal and soya concentrate were significantly lower ($p<0.05$) than the control group. The same trends were also observed for protein efficiency ratio and apparent net protein utilization. Protein digestibility coefficients were lower for the overheated and underheated full fat soya meals and soya concentrate as well as for the solvent extracted product compared to the control and properly heated soyabean groups.

4.3.2. MATERIALS AND METHODS

4.3.2.1. Experimental fish and feeding regime

Gilthead seabream of mean weight 1.61g were obtained from the Cefalonia hatchery in Greece. The fish were treated as in section 3.5.2.1. and left to acclimate for one month. Seventeen fish were stocked in each of the rearing tanks and the fish were fed in duplicate the experimental diets at a fixed ration of 4% of body weight daily. All the experimental procedures followed were similar as described in other sections.

A group of forty fish having a mean weight of 170g were distributed into four digestibility chambers for collecting faeces in order to determine the protein digestibility coefficients of the experimental diets. Fish were fed a fixed ration of 2% of body weight daily for nine days and the faeces pooled and analyzed as described in an earlier section (section 2.3.3.).

4.3.2.2. Experimental facilities

The experimental trial was conducted in the same experimental unit described in section 3.5.2.2. and all the environmental conditions were maintained at the same levels.

The digestibility trial took place in the same rearing system described in section 2.3.2. and all the procedures

followed, were identical as described previously.

4.3.2.3. Experimental diets

Six balanced diets were formulated in which white fish meal was used as the major dietary protein source (table 30). In all diets the different soyabean meals replaced 35% of the crude protein, except for the control mix where all the protein was of fishmeal origin. All the diets were designed to have the same crude protein content (45%), oil (12%) and gross energy (18 MJ/Kg).

The soyabean meals under investigation were:

1. A full fat soyabean meal which was heated to 150^{o} C and cooked for 5 minutes at 110^{o} C (light cook, LS).
2. The same meal heated to 150^{o} C and cooked for 20 minutes at 110^{o} C (standard cook, SS).
3. The same meal heated to 150^{o} C and cooked for 45 minutes at 110^{o} C (heavy cook, HS) (All these materials were Kindly prepared by Cherwell Valley Silos Ltd, Banbury, England)
4. A standard solvent extracted soyabean meal (SES) and
5. A commercially developed soy protein concentrate (Danpro-A, Arhus Ollie ltd, Denmark) derived from dehulled soyabeans (DAN).

Vitamin and mineral premixes were added to provide for the nutritional requirements of seabream. Supplemental oil and carbohydrate sources were added as before. Chromic oxide

Table 30. Formulation of experimental diets for seabream using differently processed soyabean meals.

	DIET 1 FM 100%	DIET 2 LS 35%	DIET 3 SS 35%	DIET 4 HS 35%	DIET 5 SES35%	DIET 6 DAN35%
White fishmeal	71.9	46.74	46.74	46.74	46.74	46.74
Full fat soyameal (LS)	-	42.53	-	-	-	-
Full fat soyameal (SS)	-	-	45.68	-	-	-
Full fat soyameal (HS)	-	-	-	44.28	-	-
Soyabean meal (SES)	-	-	-	-	36.58	-
Danpro-A (DAN)	-	-	-	-	-	23.63
Cod liver oil	6.97	0.16	-	-	8.07	8.61
Starch/Dextrin	10.00	1.02	2.04	0.70	-	5.94
Ascorbic acid	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	-	2.44	2.44	2.44	2.44	2.44
ℭ-Cellulose	8.03	4.01	-	2.74	3.07	9.54
Binder (guar gum)	0.50	0.50	0.50	0.50	0.50	0.50
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50
NUTRIENT ANALYSIS (%)						
Moisture	5.63	8.56	5.75	5.32	5.38	5.56
Protein	44.08	45.02	46.88	45.23	45.08	45.18
Digestible protein	39.88	38.16	40.12	37.85	37.58	37.14
Lipid	12.78	12.14	12.69	12.62	12.48	12.35
Ash	10.03	14.11	13.12	12.15	12.01	10.93
Fibre	9.11	9.69	5.10	7.98	8.35	12.41
NFE	18.38	13.18	16.46	16.70	16.70	13.57
Energy (MJ/Kg)	17.87	18.28	17.62	17.82	17.76	17.88

NFE Nitrogen Free Extracts calculated by difference

Table 31: Amino acid profile of diets containing inclusion of various soyabean meal products (%of diet).

	FM 100%	LS 35%	SS 35%	HS 35%	SES 35%	DAN 35%
Methionine	1.51	1.00	0.98	0.99	1.05	1.00
Met+Cysteine	1.82	1.58	1.54	1.59	1.67	1.61
Lysine	3.21	3.03	3.12	3.11	3.18	2.57
Tryptophan	0.42	0.55	0.52	0.54	0.55	0.46
Arginine	3.19	3.15	3.14	3.17	3.35	2.91
Histidine	1.01	1.33	1.29	1.32	1.38	1.29
Threonine	2.11	1.93	1.87	1.90	1.95	1.70
Isoleucine	1.81	2.37	2.41	2.28	2.40	2.00
Leucine	2.93	3.29	3.34	3.46	3.49	3.20
Valine	2.14	2.38	2.40	2.29	2.51	2.15
Phenylalanine	1.59	2.20	2.20	2.14	2.30	1.71

(Cr_2O_3) was incorporated at a level of 0.5% in the diet in order to facilitate the determination of the digestibility coefficients.

The weighed dietary ingredients were mixed, pelleted, dried and stored at -20° C before being presented to the fish.

4.3.2.4. Analytical techniques

The same chemical determinations described so far were employed for this present study.

The method used for trypsin inhibition activity (TIA) was the one described by Kakade et al., (1974) and it was slightly modified by the author.

Trypsin Inhibition Activity (TIA)

The samples were defatted, dried and powdered to pass through a 100-mesh sieve. 0.5g of the sample were accurately weighed, placed in a 50 ml conical flask and 25 ml of 10 mM NaOH were added. The sample was macerated for 30 seconds, the pH adjusted between 9.4 and 9.6 with 1 M NaOH or 1 M HCl, and left for four hours at ambient temperature with continuous stirring and stored overnight at -4° C.
After extraction, the suspension was diluted D times in order to produce inhibition within the range of 40-60%. The following were then pipetted into a series of 10 ml tubes:

a. reagent blank : 1 ml distilled water

b. standard (20 mg trypsin) : 1 ml standard trypsin solution and 1 ml distilled water,

c. sample blank : 0.5 ml diluted sample extract and 0.5 ml distilled water and

d. sample : 0.5 ml diluted sample extract and 0.5 ml of distilled water.

After mixing and preheating to 37^{o} C for 15 minutes, 2.5 ml BAPNA solution (40 mg BAPNA and 1 ml dimethyl sulphoxide were diluted to 100 ml with Tris buffer, previously warmed to 37^{o} C) were added into each tube and mixed. After exactly 20 minutes incubation at 37^{o} C, each tube received 0.5 ml of acetic acid (30% V/V) to stop the reaction. Standard trypsin (1ml) was then added to reagent blank and sample blank tubes. After centrifuging the absorbance was measured at 410 nm. The mg of the trypsin inhibited per gram of sample was calculated using the formula:

$$TIA = \frac{1.25 * D * A_I}{S}$$

where,

A_I : (Ast-Arb)-(As-Asb), where Ast, Arb, As and Asb is the absorbance of standard, reagent blank, sample and sample blank respectively.

D : The dilution factor

S : Sample weight (g)

Cresol red

This method was used as described by Olomucki and Bornstein (1960) and directly measures the degree of soybean meal processing.

According to the method 400 mg of ground meal were weighed into 50 ml polypropylene tubes. 10 ml of working dye solution (1 part stock dye solution ie, 0.2% cresol red in alcohol, and 9 parts of 0.1 N HCl) were added, the tubes closed and stirred for one hour. The samples were then centrifuged for 10 minutes at 10000 rpm, the supernatant removed and transferred into a 15 ml polypropylene tubes for a further 10 minutes of centrifugation at 10000 rpm. 10 ml of 0.02 N NaOH were added to 1 ml of the supernatant product and the colour intensity measured at 570 nm using a background correction at 640 nm. A standard calibration curve was prepared using 0.1-0.5 ml of the working dye solution in 0.02 N NaOH (final volume 11 ml). The mg of dye per gram of the meal were calculated by the formula:

$$\text{mg dye/g meal} = \frac{2000 - \text{conc} * 100}{S}$$

where,

conc : Concentration of sample (mg of dye)

S : sample weight (mg)

4.3.3. RESULTS

Average body weight increase of the fish fed the experimental diets is presented in Figure 16. The growth response, feed utilization and carcass composition are shown in Table 32. Fish fed the diets containing standard cook soya (SS), heavy cook soya (HS) and the fishmeal based reference diet exhibited similar growth patterns throughout the trial period. Growth rates declined when light cook soya (LS), solvent extracted soya (SES) or Danpro-a (DAN) were incorporated into the diet. The mean weight gain of the fish fed the HS diet was the highest and was significantly higher than those fish fed the diets containing LS, SES, and DAN ($p<0.05$). However, no significant differences were identified among the weight gains of fish receiving the diets containing fishmeal alone or SS and HS substitution. The value obtained for gilthead bream fed the LS diet was lower than that of the SS and fishmeal groups. This difference was not, however, significant ($p<0.05$). The lowest weight gain was recorded by the fish fed the DAN soy protein concentrate containing diet. Specific growth rates and feed efficiency followed similar trends. Protein efficiency ratio was significantly higher for the groups fed the control and HS diets and declined to the lowest value estimated for the DAN fed fish. Apparent net protein utilization was highest for the HS group but not significantly higher than the values obtained from the

Table 32. Growth performance and feed utilization of seabream fed experimental diets containing various soyabean meal products.

	DIETS						
	FM 100%	LS 35%	SS 35%	HS 35%	SES 35%	DAN 35%	+SEM[1]
Initial mean weight (g)	1.61[a]	1.62[a]	1.65[a]	1.56[a]	1.63[a]	1.56[a]	0.12
Final mean weight (g)	11.75[b]	11.11[ab]	12.21[b]	12.23[b]	10.67[ab]	9.70[a]	0.48
Weight gain (%)	629.10	587.56	641.15	683.96	553.73	522.16	
Specific growth rate (%/day)	2.36[bc]	2.30[ab]	2.38[bc]	2.45[c]	2.23[a]	2.18[a]	0.22
Food intake (mg/day)	180.14	178.17	182.49	187.57	170.39	156.16	
Weight gain (mg/day)	120.65	112.98	125.71	126.96	107.62	96.85	
Feed efficiency	69.93[bc]	63.41[ab]	68.89[c]	67.68[c]	63.07[a]	62.00[a]	1.10
Protein efficiency ratio	1.43[c]	1.33[ab]	1.39[bc]	1.42[c]	1.32[ab]	1.28[a]	0.02
Nitrogen intake (mg/day)	84.11	85.24	90.71	89.58	81.19	75.89	
Nitrogen deposition (mg/day)	20.04	18.93	21.68	23.13	17.70	16.52	
Apparent net protein utilization	23.85[ab]	22.19[a]	23.90[ab]	25.81[b]	21.80[a]	21.76[a]	
CARCASS ANALYSIS (% wet weight)							
	Initial Fish						
Moisture	74.47	70.77[a]	70.85[a]	69.13[a]	68.66[a]	70.51[a]	69.82[a]	0.77
Protein	16.70	16.66[a]	16.74[a]	17.17[a]	18.02[a]	16.52[a]	17.00[a]	0.44
Lipid	2.72	8.36[a]	7.80[a]	9.58[a]	8.74[a]	8.65[a]	8.56[a]	0.63
Ash	5.53	4.47[a]	4.41[a]	4.57[a]	4.68[a]	4.39[a]	4.47[a]	0.15

Figures with common superscripts in each horizontal row are not significantly different ($p < 0.05$).

[1]SEM Standard Error of the Mean calculated from the residual mean square in the analysis of variance.

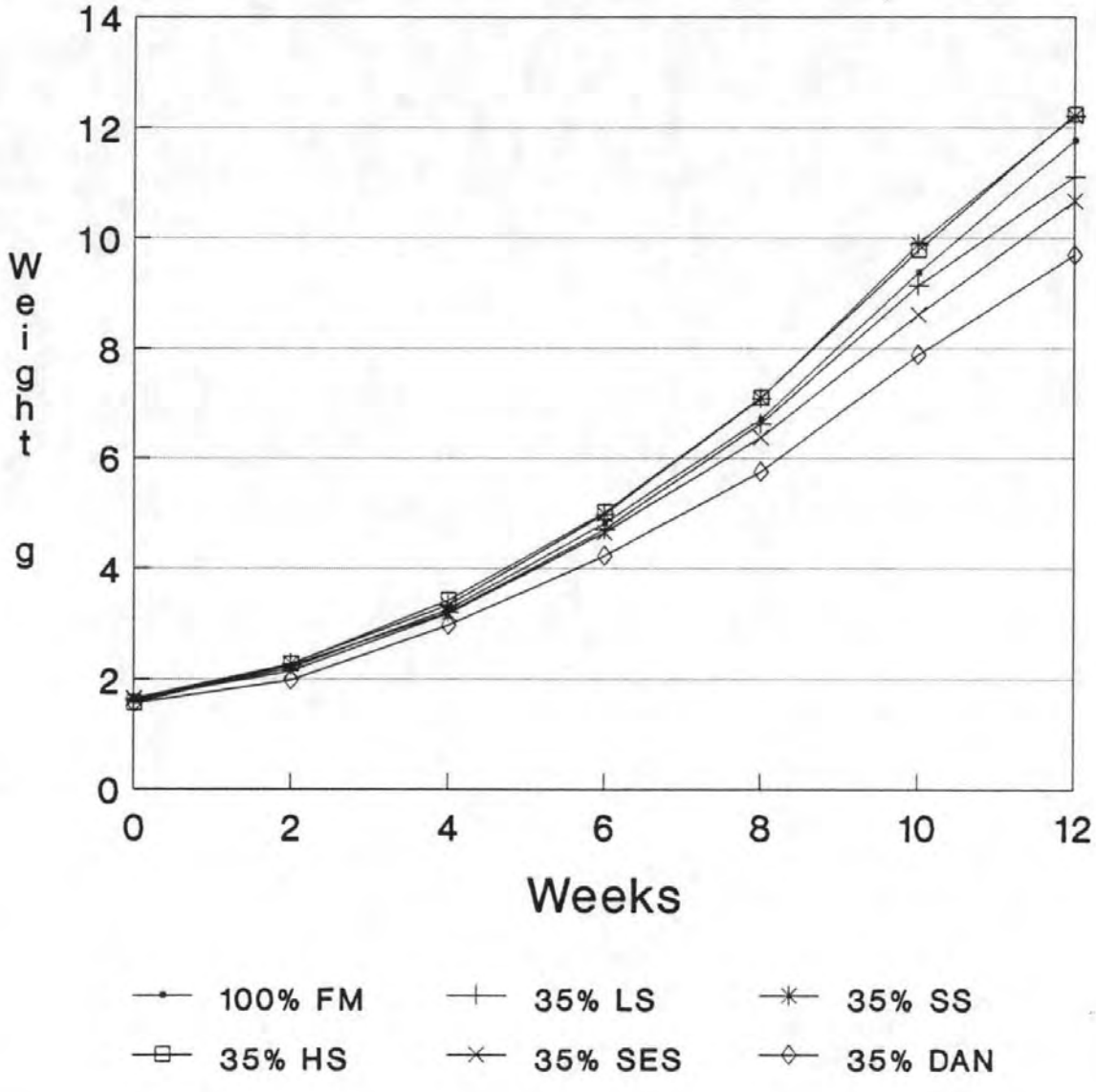

Figure 16. Mean weight increase of sea bream fed diets containing different soyabean products.

control and the SS formulations.There were no significant differences ($p<0.05$) in the percentage carcass composition in the fish for each treatment. A considerable lipid increase was observed between the initial and final population but this might be expected for seabream receiving high energy diet formulations.

Protein digestibility coefficient was highest for the control diet followed by the SS containing group. A slight reduction of the digestibility coefficients was observed for all the remaining experimental formulations. Digestible crude protein (DCP) values of the diets are presented in Table 30.

Table 33 summarizes the results for available lysine, trypsin inhibition activity and cresol red values. According to the findings, heating did not decrease the lysine availability of the meals significantly. The DAN product showed the lowest value. Cresol red estimations confirmed the trends obtained with the available lysine determinations on the same materials. According to Olomucki and Bornstein (1960), the range of the meals may be classified as underheated (cresol red values, 2.7-3.7), properly heated (cresol red values, 3.8-4.3) and overheated meals (cresol red values, above 4.3). In the present study, the products may be ranked as :

LS, SS, SES : underheated

HS : properly heated

DAN : overheated

Table 33. Trypsin Inhibitor Activity (TIA), Cresol red values and "available" lysine estimations of the soyabean products tested.

	TIA (mg trypsin inhibited/ g of meal)	%TIA destroyed	Cresol red (mg dye/ g of meal)	"Available lysine" (%protein)
Raw full fat soya meal	20.83		1.75	7.15
Full fat soya meal (LS)	6.92	66.93	3.43	6.52
Full fat soya meal (SS)	4.39	79.03	3.66	6.51
Full fat soya meal (HS)	3.10	85.19	3.96	6.21
Soya meal (Solvent ext.)	3.49	83.33	3.66	6.46
Danpro-A	3.05	85.42	4.79	5.56

Trypsin inhibition was reduced with increased heating and the DAN meal exhibited the lowest value for the products tested.

It was concluded that a properly heated full fat soyabean meal is utilized by seabream better than the standard solvent extracted type meal at the levels tested. The most discouraging results were yielded by the soy protein concentrate meal and this was probably because of the relatively low lysine availability observed for this material.

4.4. DISCUSSION

The present investigation showed that at levels contributing somewhere in the region of 20-30% of total dietary protein, solvent extracted soybean meal causes some depression of the growth response of juvenile gilthead seabream. In contrast Aurelio et al. (1988) found that at levels up to 29% of the diet, soybean can effectively replace fishmeal without significant growth decrease when fed to seabream (Sparus aurata). Studies with Tilapia (Sarotherodon) have shown equivalence of soybean meal as partial replacement for fishmeal at a level of 20% (Viola and Arieli, 1983). Jackson et al. (1982) identified a decrease of growth when more than 25% of soybean was included in the diets of Sarotherodon mossambicus. Shiau et al. (1987) agreed with these findings for the same species. In a later study the latter authors (Shiau et al., 1990) suggest that defatted soya bean meal can be used to replace 30% of fishmeal protein in tilapia diets containing 24% crude protein. Dabrowski et al. (1989) found that the growth rate of rainbow trout was reduced significantly when 50% of the fishmeal was replaced by soyabean meal, and that 100% replacement resulted in growth arrestment and mortality. A decreased amino acid absorption was noticeable even when 25% of fishmeal protein had been replaced by soyabean meal protein. Mohsen and Lovell (1990) experimenting on channel catfish (Ictalurus punctatus)

observed a significant growth reduction when more than 50% of the dietary protein came from soyabean. Similarly, Ina et al. (1981) observed a reduction of both the growth and feed efficiency of red sea bream (Chrysophrys major) when they were fed experimental diets containing more than 15% soyabean meal.

Rainbow trout, however, successfully utilized soybean inclusions replacing up to 75% of the protein with no obvious changes in overall growth performance and feed utilization (Tacon et al., 1983). In agreement are the findings of Reinitz (1980) who fed a diet containing 65% soybean meal to rainbow trout and the fish grew at an acceptable rate and remained in good health. Further confirmation that growth rate, feed efficiency and mortality were not affected by incorporating soyabean meal at a level of 39% of the diet for rainbow trout was obtained by Cho et al. (1976).

On the whole, soyabean meal provides a fairly balanced gross amino acid profile, but is relatively deficient in methionine and lysine when compared to high quality fish meals. Many workers have found that supplementation of these amino acids improved the performance of the diet (Siau et al., 1987). Dabrowski and Wojno (1977) found that rainbow trout can utilize soyabean protein enriched with cysteine (1%) and tryptophan (0.5%) almost as well as protein from fishmeal. Murai et al. (1989) fed carp fingerlings (Cyprinus carpio) on a 40% soyabean diet supplemented with 0.25%

tryptophan and an essential amino acid mixture. These workers reported enhanced growth rates and feed efficiency on such diets. Viola et al. (1982) found that 0.15% of methionine was sufficient within a diet containing 40% soyameal for carp in order to maintain growth at a similar level to fish fed a fishmeal control diet. In addition, 0.5% methionine and 0.5% lysine added to a 100% soyameal diet supported growth rates almost equal to that of the control group receiving fishmeal as the main protein source.

As stated previously, soyabean also contains several antinutritional factors which can be inactivated by heat treatment. Commercial solvent extracted soyabean can still retain these factors due to insufficient heat application. Due to improper processing, the carbohydrate fraction of the material will also contain large amounts of indigestible oligosaccharides.

New processing technology methods have resulted in a series of new soyabean meal materials and products. These now include full fat soyabean meals of high energy value, and concentrate feed ingredients such as the Danish DANPRO soy range (crude protein 66%) and dehulled products (HYPROSOY, CP-49%).

The results of the present investigation showed that standard solvent extracted soybean meal can only be used to substitute not more than 20-30% of the diet for the gilthead seabream without the support of any amino acid

supplementation.

A threshold level of 35% replacement of dietary protein was therefore chosen for all comparisons in order to assess improved nutritional value as a result of processing.

Sparus aurata efficiently utilized the full fat soya meals at the level of 35% of the dietary protein. The study showed that a properly heated full fat soyabean meal can be included at this level with no impairments on growth performance.

Reinitz et al., (1978) fed diets containing 73% full fat soya meal to rainbow trout (Oncorhynchus mykiss). The fish fed the full fat meal formulations outperformed the fish fed the standard diet containing 25% herring meal and 20% defatted soyabean meal in terms of average weight gain, feed efficiency and daily length increment. Tacon et al., (1983) observed no adverse effect on growth performance of rainbow trout fed diets containing full fat soya meal at levels up to 75% of the protein component. Smith (1977), observed a significant reduction on weight gain when rainbow trout were fed a diet containing 80% solvent extracted soybean meal compared to the gain of fish fed a formulation containing 80% full fat soya meal. Channel catfish fed diets containing full fat soyabean meal, however, gained the same amount of protein and much more fat than fish fed diets containing solvent extracted soya bean meal (Lovell, 1985). Catfish do not appear to benefit as much as trout from full fat soyabean meals. Similar findings are reported by Shiau et al. (1990)

who fed tilapia fingerlings on diets containing defatted and full fat soya meals. The data obtained by these authors suggest that either meal can be used to replace 30% of the fishmeal protein. It has been stated that the additional fat contained in the full fat soyabean is probably less beneficial to warm water fish than cold water fish, since warm water species like catfish, carp and tilapia can utilize the less expensive carbohydrates from grain sources relatively well for energy (Lovell, 1980).

The soyabean protein concentrate exhibited the lowest growth and feed utilization. In agreement to these findings Alexis (unpublished data) observed a decreased growth performance when rainbow trout was fed with a high protein soya concentrate. Contradictory results are reported by Davies et al. (1989) who used Danpro-A on tilapia (O. mossambicus) at levels up to 75% of the dietary protein with no adverse effect on growth parameters.

In the present investigation, the growth of sea bream was improved with increased heat treatment for the full fat soya meal. Fish fed the HS meal, characterized by a cresol red value of 3.96 and trypsin inhibition activity (TIA) of 3.10 , grew best. The SS diet having a cresol red value of 3.66 and TIA of 4.39, resulted in only a slight growth reduction. When the TIA was estimated at a value of 6.92 for LS meal, the adverse effect on growth was significant. Danpro-A gave the highest cresol red value of 4.79 and the lowest TIA of 6.10.

Lysine availability was fairly similar for all meals except for the DAN meal which exhibited the lowest value. Viola et al. (1983) reported that the growth rates of common carp (Cyprinus carpio) were reduced when fed diets containing insufficiently heated soyabean meal. The latter authors observed similar growth rates in carp fed diets containing properly heated and slightly overheated soyabean meals. They concluded that the main limiting factor of growth of carp was not TIA but inadequate lysine availability. The properly heated and slightly overheated meals used in these latter studies had 95-100% of the TIA destroyed. Wilson and Poe (1985a), observed that channel catfish can utilize soyabean meal with higher TIA than carp. Even though growth rates and PER values reported for the study were not significantly different over a wide range of TIA, maximum growth rates were observed when about 83% of TIA in the soyabean meal had been destroyed. In the present study an 80% destruction of TIA in the soyabean meal proved sufficient for the growth of seabream. Properly heated and underheated meals all proved to be good sources of lysine.

To summarize, the results of the combined experiments exploring the possibility of using selected animal and plant

byproducts as feedstuffs for the gilthead seabream have proved quite encouraging. However, realistic appraisals must be made for any farmed fish with respect to practical diet formulations. This objective formed the basis for the next investigation.

CHAPTER 5

5. EXPERIMENT 8

FORMULATION OF PRACTICAL TYPE DIETS FOR THE GILTHEAD SEABREAM SPARUS AURATA

5.1. INTRODUCTION

Practical diet formulation represents the translation of energy and nutrient requirements into a balanced blending of feed ingredients designated for a specific fish species (Cho et al., 1985).

The feed ingredients are chosen on the basis of gross nutritional profile, obtained by the application of relevant analytical techniques; the nutrient requirements of the fish, reported from in vivo investigations for that species; the availability of nutrients to the fish from the various feed ingredients determined from digestibility and growth trials; and the minimum-maximum levels of inclusion levels of the feedstuffs according to physical characteristics; and final cost (Lovell, 1989).

Data on the nutrient contents of all common fish feed ingredients are available in feed composition tables found in specialized publications (ADCP, 1983; Tacon, 1987). These values, however, are only averages since ingredients vary

considerably in nutrient content depending on the source and type of processing. Also, analytical methods are usually subject to experimental error and variability between laboratories (Jobling, 1983). As a consequence feeds are often formulated to contain nutrient values in excess to those actually desired (Hardy, 1989). Another very important aspect in feed formulation is the availability of nutrients in a feed ingredient. Such factors as the processing technology applied on the material as well as interactions with other dietary ingredients can reduce the availability of specific nutrients to the fish (Crampton, 1985).

Upper, lower or fixed limits in which the level of a specific ingredient is included in a mixture are often set in the diet formulation procedure. Upper limits are placed on ingredients containing either antinutritional or toxicant factors or due to palatability and pelletability effects. Lower limits are set when feed ingredients are shown to be indispensable sources of nutrients and hence are included irrespective of their cost (Waldroup, 1984). Forced inclusions are usually for vitamin and mineral premixes or for some ingredients with desirable properties, beneficial in the finished feed.

The process of practical diet formulation is largely a compromise between two major objectives. One is to formulate primarily on economic considerations and the other to meet the specific nutritional requirements of the fish species in

question.

Formulating on a least cost basis will usually result in less expensive but nutritionally inferior products. Placing nutritional value as a priority will, on the other hand, produce a more expensive feed that is more productive in terms of growth performance and feed utilization.

However, for effective feed formulation, sufficient knowledge of the cost of the feed per unit of production should be employed. In other words, the market value of a fish species determines the relative importance of feed efficiency and the quality of the product required. This can, of course, vary depending upon the cost of feed in relation to the value of the fish (Blake, 1987).

The procedure of diet formulation is outlined in Figure 17 according to Cho et al. (1985). First the energy needs of the fish species must be estimated from information on maintenance requirements and growth; then the protein and amino acid needs are estimated according to the energy content of the diet (step 1). The next step is to select ingredients that are locally available and which meet the requirements of the fish (step 2). The basal part of the feed can then be designed and mixed with the addition of oil to provide part of this energy concentration and the essential fatty acids (step 3). Usually, fixed levels of vitamin and mineral premixes are also included. These are always formulated separately on the basis of specific information

for the fish species under consideration or at levels providing a safety margin in excess of the requirements estimated (step 4). Filler type ingredients are also included in the form of cereals or grain derived products to provide some degree of supplementary energy and dietary bulk (step 5). Following this protocol and using the data extracted from steps 1-5 the final diet formula and composition are obtained (step 6).

It should be realized that continuous quality control of the compounded feeds is essential in order to monitor the consistency of the final product.

In the past, simplistic attempts were made to design diets for farmed animals when only a few raw materials are considered. Early attempts made use of the Poulson square approach to solve linear equations for protein level and energy concentration in a complete diet (Patrick and Shaible, 1980).

Advances in feed technology and nutrition research demand more complex considerations in the design of animal feeds.

The acceptance of linear programming in the formulation of least cost diets for livestock and poultry feeds has been extremely rapid and is attaining almost universal usage in the feed industry. The role of the personal computer (PC) and the development of PC compatible software for such purposes has enabled research institutes and the smaller feed compounder to use these techniques. Some least cost formulae

have been employed successfully within the commercial aquaculture sector for several years (Chow et al., 1980).

The only difference in feeds for aquaculture is that the amount of information on nutrient availability and ingredient restriction for many fish species, is sometimes contradictory and incomplete (Cho et al., 1985).

Linear programming is essentially a mathematical technique that determines the optimum allocation of resources (in our case, different feedstuffs) to obtain a particular objective (such as reducing cost) when there are alternate uses for the resources or if selection can be made from among the resources available (Waldroup, 1984).

In addition to formulating the basic least cost diet, linear programming has a number of uses in feed formulation. The technique can be used to analyze the economics of the alternate availability of resources or to explore the effects of changes in nutrient specification (Crampton, 1985). In this manner, it may be used by the fish nutritionist as a useful tool for both research and experimentation.

In the present study, least cost formulation software was employed for the computerized design of feeds. This approach was used in order to design practical type diets for the gilthead seabream. Restrictions and limitations regarding the inclusion of feed ingredients were made according to some

of the data obtained from the experiments reported in the previous sections. Slight modifications were made, where necessary, for practical and technical reasons.

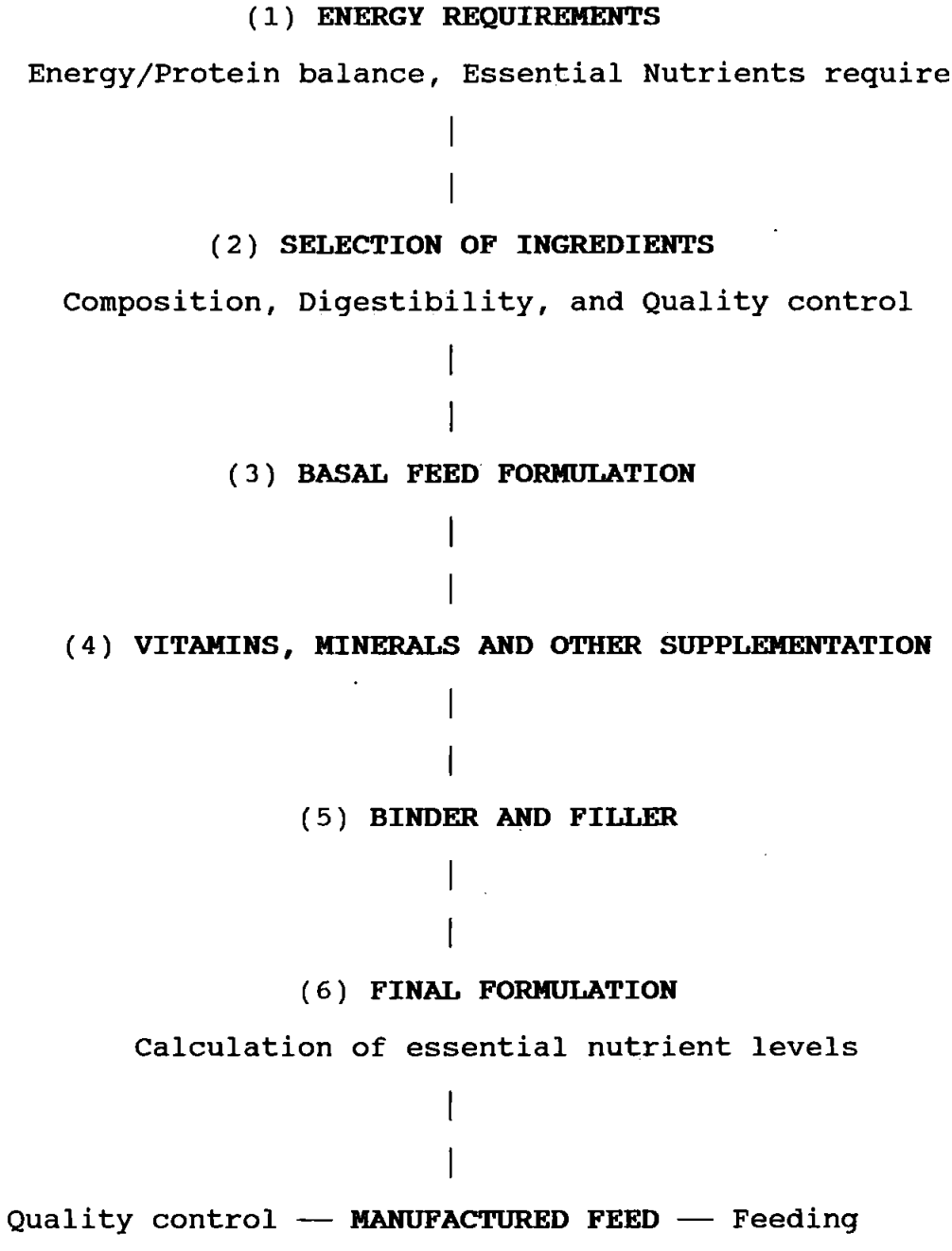

Figure 17. Schematic outline of the fundamental considerations required for the practical formulation of diets for farmed fish.

5.2. ABSTRACT

Sea bream (Sparus aurata) of approximately 1.3g average initial weight were fed nine practical type diets together with a commercial diet for 70 days. All the diets had the same proximate composition set at 45% crude protein, 15% crude lipid and 20 MJ/Kg gross energy on an as fed basis. The tested rations were formulated either by the partial or complete substitution of fishmeal by poultry meat meal, meat and bone meal, full fat soya meal and wheat middlings. Apart from one formulation containing a high poultry meat meal level, the performances of all diets were shown to be significantly inferior when compared to both the control and commercial feeds. A significantly negative correlation was also observed between the dietary full fat soya meal levels and fish growth and feed utilization. Feed efficiency ranged from 94 to 67%, specific growth rate from 2.33 to 1.86, protein efficiency ratio from 1.7 to 1.29 and apparent net protein utilization (NPU) from 28.76 to 31.19.

In addition protein digestibility coefficients (DCp) were also determined for the range of the tested diets. All coefficients were found to be relatively high for the mixed protein sources in the diets.

5.3. MATERIALS AND METHODS

5.3.1. Experimental fish and feeding regime

Gilthead seabream having a mean initial weight of 1.31g were transported from Cefalonia hatchery to the National Institute for Marine Research (NCMR) in Greece. The fish were treated and left to acclimate as described in previous experiments.

Fifteen fish were stocked in each 30l tank and the ten diets were fed in triplicate. The feeding level was set at 4% of body weight per day and the amount was adjusted every two weeks, following weighing and routine inventory, during the 70 day period of the study.

Initial and final populations were sacrificed and analyzed for gross carcass analysis.

A group of forty fish averaging 120g was employed in a separate study to determine the protein digestibility coefficients for each of the experimental diets. The seabream were fed at a level of 2% of body weight per day for nine days, the faeces pooled at three day intervals and treated according to a previously described protocol.

5.3.2. Experimental facilities

The growth experiment took place in the same sea water rearing system described in section 3.5.2.2. All

environmental water parameters were held once again at the same levels as with previous experiments (temperature, 22^{o} C) and were monitored routinely.

The digestibility conical tank system used for the determination of protein coefficients were also the same as that described before. Identical procedures were followed regarding the collection and treatment of the faecal material as well as the daily maintenance of the experimental conditions.

5.3.3. Experimental diets

Nine practical type diets were formulated and each one was assigned to three groups of fish. A commercial feed (Aqualim SA, France) was also used for comparison. The proximate composition of the ingredients used in the formulation of the diets is given in Table 34. The composition of each of the diets as well as their nutrient analysis are displayed in Table 35.

The practical rations were designed using a least cost formulation computer programme (ULTRAMIX) developed by Alan Munford (Exeter University, 1988). Two groups of diets were designed; a low fishmeal series (20% of dietary protein) and a high fishmeal range (50% of the protein). For each diet, a forced inclusion level of one of the ingredients was set, based on several of the findings obtained from the previous

Table 34. Nutrient composition of raw materials used in the practical feed formulation study.

	(% as recieved)						"Available" Lysine		
	Moisture	Protein	Lipid	Ash	Fibre	NFE	(% protein)	TIA	Cresol red
White fish meal	2.82	66.18	8.63	21.12	2.55	-	6.54		
Meat and bone meal (C)	2.46	59.35	8.90	24.84	5.13	-	3.54		
Full fat soya meal	14.38	34.48	19.31	4.51	11.31	17.42	6.49	4.06	3.81
Poultry meat meal (PMM)	5.00	62.00	13.50	15.00	2.00	-	5.43		
Wheat middlings	11.00	17.70	3.60	5.50	7.00	60.00	-		

NFE calculated by difference

Table 35. Formulation of practical type diets for seabream showing percent replacement of protein with byproducts.

	DIET 1 FM100%	DIET 2 FM 20% SOYA 20%	DIET 3 FM 20% MBC 25%	DIET 4 FM 20% PMM 50%	DIET 5 FM 50% SOYA 30%	DIET 6 FM 50% SOYA 20%	DIET 7 FM 50% MBC 20%	DIET 8 FM 50% PMM 30%	DIET 9 FM 0%	DIET 10 COMMERCIAL[1]
White fish meal	68.60	16.00	16.00	16.00	37.80	37.80	37.80	37.80	-	*
Meat and bone meal (C)	-	15.60	25.00	9.60	6.90	9.50	19.00	6.60	30.10	*
Poultry meat meal(PMM)	-	24.00	27.10	39.90	5.00	5.00	5.60	23.90	43.70	*
Full fat soya meal	-	32.20	7.30	11.60	40.50	32.20	15.50	-	-	*
Wheat middlings	17.70	5.70	16.10	14.40	3.00	7.40	12.10	18.30	16.70	*
Molasses	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*
Cod liver oil	1.10	2.80	4.80	4.80	3.20	3.00	3.00	3.00	5.00	*
Animal fat (lard)	8.80	-	-	-	-	1.30	3.40	4.60	0.90	*
Methionine	0.50	0.56	0.56	0.60	0.49	0.52	0.50	0.70	0.60	*
Vitamin premix	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	0.50	*
Mineral premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*
NUTRIENT ANALYSIS (%)										
Moisture	7.40	7.60	8.10	8.20	6.90	7.30	7.21	7.20	7.10	10.00
Protein	44.30	47.20	47.57	47.65	46.72	46.11	46.65	47.19	48.19	52.00
Digestible protein	40.44	39.00	39.25	41.06	39.67	39.14	39.10	41.74	39.24	-
Lipid	15.00	14.80	13.86	15.00	14.88	14.92	15.06	15.31	15.00	12.00
Ash	14.63	12.74	15.00	13.19	12.55	13.08	15.00	14.62	15.19	12.00
Fibre	2.78	5.03	4.06	3.80	5.30	5.01	4.28	3.11	3.61	1.00
NFE	15.89	12.67	11.41	12.16	13.54	13.50	11.87	12.57	10.91	13.00
Energy (MJ/Kg)	19.70	20.15	19.46	20.01	20.30	20.12	19.81	19.98	19.89	-

NFE Nitrogen Free Extracts calculated by difference

[1] Commercial marine fish diet (Aqualim, closed formula)

experiments. The linear programme was left open to decide on the inclusion levels of the remaining of the raw materials and by-products in order to satisfy the nutrient requirements set for the seabream. The relative cost of each ingredient was the driving factor determining the outcome of the formulation. An update price index was provided by Cherwell Valley Silos ltd., using the British Telecom Prestel information system at the date of formulation.

A fishmeal free diet was also included for comparison. Wheat middlings was used as the principle carbohydrate source in all of these diets. Marine oil was supplemented at a set level to provide adequate amounts of the n-3 fatty acids required by bream. Lard was included where necessary to maintain a similar ratio of animal fat to marine oil in all the diets. Sugar beet molasses at a level of 0.1% was also added as the binder, vitamin and mineral premix formulations were included as before and finally D.L.methionine was supplemented to all diets to prevent any limitations for this amino acid. Amino acid profiles of the mixtures are presented in Table 36.

The feeds were mixed and pelleted as in all other trials. Before pelleting, a quantity of the mixture was separated, chromic oxide (Cr_2O_3) was added at 1% of the diet and pellets were made for the subsequent digestibility experiment.

All the diets were designed to be isonitrogenous (45% crude protein), isocalorific (20 MJ/Kg gross energy) and to

Table 36. Amino acid profile of practical type diets for seabream (% of diet).

	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6	Diet7	Diet8	Diet9	Diet10
Methionine	1.67	1.51	1.55	1.56	1.54	1.56	1.58	1.76	1.53	1.34
Met+Cysteine	2.05	2.00	2.01	1.98	2.03	2.00	1.92	2.12	2.01	1.82
Lysine	2.74	2.87	2.79	2.74	2.98	2.90	2.89	2.72	2.72	3.66
Tryptophan	0.35	0.44	0.40	0.40	0.46	0.44	0.41	0.36	0.38	0.52
Arginine	2.79	3.04	2.91	2.86	3.15	3.06	3.01	2.78	2.83	3.12
Histidine	0.93	1.00	0.94	0.90	1.07	1.04	1.01	0.89	0.87	1.52
Threonine	1.72	1.90	1.82	1.81	1.94	1.89	1.85	1.75	1.80	2.19
Isoleucine	1.58	1.97	1.77	1.78	2.03	1.94	1.82	1.64	1.72	1.91
Leucine	2.70	3.22	3.17	3.10	3.15	3.09	3.09	2.90	3.23	3.85
Valine	2.23	2.21	2.09	2.16	2.37	2.30	2.21	2.16	1.98	2.66
Phenylalanine	1.44	1.93	1.75	1.72	1.94	1.85	1.76	1.54	1.73	2.06

contain 15% crude lipid as far as possible.

5.3.4. Analytical techniques

All the chemical analysis used in this trial were the same as the ones described in relevant previous sections.

5.4. RESULTS AND DISCUSSION

The growth characteristics of the fish over the experimental period are presented in figures 18 and 19. Performance factors such as growth rate, feed utilization parameters and body composition of the fish are reported in table 37.

The groups fed the control ration (diet 1), the commercial feed (diet 10) and the high fishmeal, high poultry meat meal diet (diet 8) grew significantly better than all the other fish ($p<0.05$). The fish fed the diets 2 (low fishmeal, high full fat soya meal), diet 5 (high fishmeal, high full fat soya meal), and diet 6 (high fishmeal, low full fat soya meal), yielded the lowest weight gains. All these formulations contained appreciable levels of full fat soya meal. A negative correlation was determined with full fat soya meal inclusion and fish growth ($r=-0.94$, $p<0.001$). Diets containing low fishmeal, high meat and bone meal (diet 3), low fishmeal, high poultry meat meal (diet 4), high fishmeal, high meat and bone meal (diet 7) and the fishmeal free formulation (diet 9) exhibited similar results which were, however, significantly lower than the control groups and diet 8. Specific growth rate (SGR) ranged from 1.86 to 2.33 following the same statistical patterns as weight gain. Feed efficiency was relatively high for the diets tested and ranged from 94.2% for the commercial feed to 67.5% for diet

Table 37. Growth performance and feed utilization of seabream fed practical type formulations.

	DIETS										
	1	2	3	4	5	6	7	8	9	10	+SEM[1]
Initial mean weight (g)	1.30^{a}	1.30^{a}	1.30^{a}	1.29^{a}	1.30^{a}	1.32^{a}	1.34^{a}	1.31^{a}	1.30^{a}	1.31a	0.13
Final mean wight (g)	8.81^{d}	6.20^{a}	7.72^{c}	7.32^{bc}	6.34^{a}	6.87^{ab}	7.50^{bc}	8.74^{d}	7.42^{bc}	9.28^{d}	0.28
Weight gain (%)	577.86	375.99	429.16	465.67	386.80	420.66	461.93	568.60	468.96	609.95	
Specific growth rate (%/day)	2.28^{d}	1.86^{a}	2.11^{c}	2.06^{bc}	1.88^{a}	1.96^{ab}	2.05^{bc}	2.26^{d}	2.07^{bc}	2.33^{d}	0.31
Food intake (mg/day)	125.56	103.74	113.35	110.95	105.79	107.46	112.13	121.03	110.09	120.86	
Weight gain (mg/day)	107.33	70.00	91.62	86.10	72.00	79.33	88.05	106.14	87.33	113.86	
Feed efficiency (%)	85.50	67.48^{a}	80.57^{bc}	77.54^{b}	68.01^{a}	73.83^{ab}	78.49^{bc}	87.64^{cd}	79.30^{bc}	94.18^{d}	2.47
Protein efficiency ratio	1.70^{d}	1.29^{a}	1.52^{c}	1.47^{c}	1.32^{ab}	1.46^{bc}	1.56^{cd}	1.70^{d}	1.54^{c}	1.54^{c}	0.05
Nitrogen intake (mg/day)	63.14	54.38	60.14	58.43	54.43	54.43	56.57	62.43	56.62	73.86	
Nitrogen deposition (mg/day)	17.88	11.52	16.08	14.45	12.40	13.50	14.78	17.97	14.46	19.95	
Apparent net protein utilization	28.35^{de}	21.19^{a}	26.61^{cde}	24.72^{bc}	22.77^{ab}	24.80^{bc}	26.10^{cde}	28.76^{e}	25.51^{cd}	26.99^{cde}	1.08
CARCASS ANALYSIS (% wet weight)											
	Initial Fish										
Moisture	74.47	67.76^{a}	69.36^{a}	68.79^{a}	68.57^{a}	67.64^{a}	68.42^{a}	68.56^{a}	67.62^{a}	69.20^{a}	67.72^{a}	0.48
Protein	16.70	16.67^{a}	16.50^{a}	17.36^{a}	16.78^{a}	17.13^{a}	16.97^{a}	16.75^{a}	16.89^{a}	16.59^{a}	17.39^{a}	0.32
Lipid	2.72	11.80^{e}	10.02^{a}	9.72^{a}	10.78^{d}	10.54^{cd}	10.23^{abcd}	10.52^{cd}	11.43^{e}	10.47^{bcd}	9.85^{ab}	0.22
Ash	5.53	3.64^{a}	4.23^{abc}	4.40^{bc}	4.00^{abc}	4.47^{c}	4.69^{cd}	4.21^{abc}	4.26^{abc}	3.75^{ab}	5.21^{d}	0.23

Figures with common superscripts in each horizontal row are not significantly different ($p<0.05$)

[1] SEM Standard Error of the Mean calculated from the residual mean square in the analysis of variance

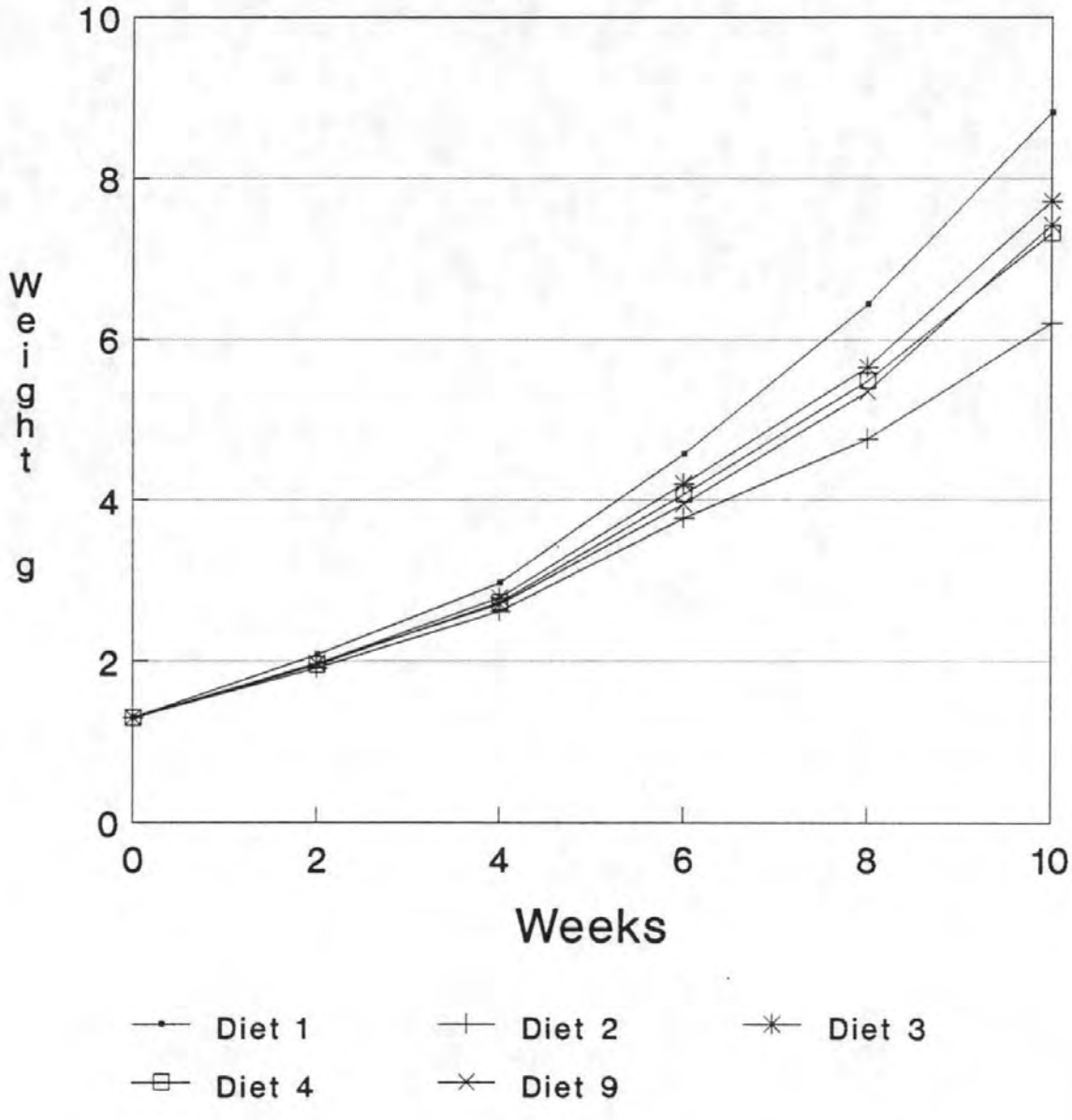

Figure 18. Mean weight increase of sea bream fed practical type diets.

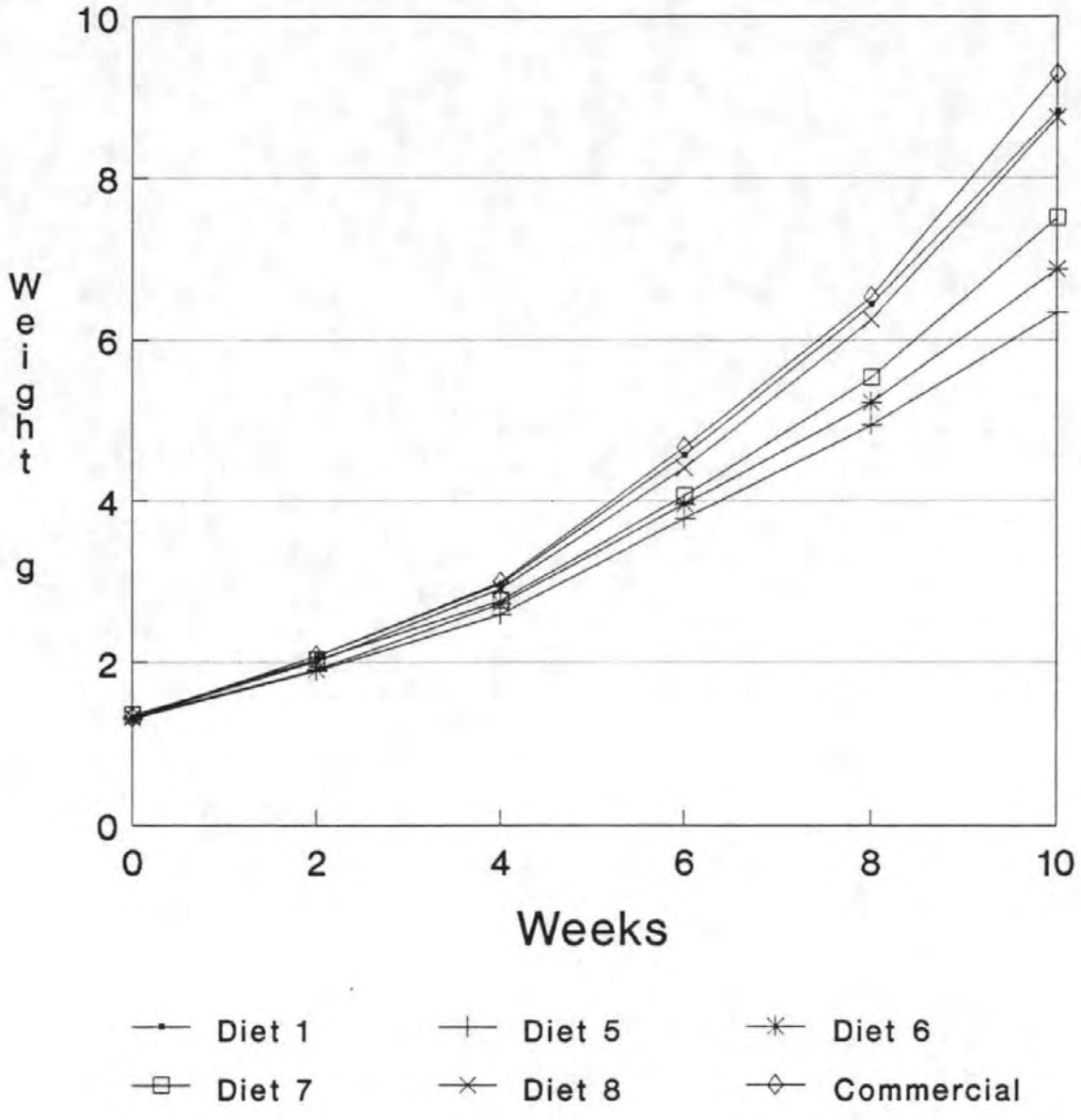

Figure 19. Mean weight increase of sea bream fed practical type diets.

2 (low fishmeal, high full fat meal). Protein efficiency ratio (PER) was significantly higher for the control formulation 1 and the diet containing high fishmeal and high poultry meat meal (diet 8). Again lowest values were obtained from the groups fed the mixtures containing high full fat soya meal inclusions. All the other diets,including the commercial ration, exhibited similar values which were only slightly inferior to the control diet 1 and diet 8. Apparent net protein utilization (NPU), supported the PER data ranging from 28.8 for diet 8 to 21.2 for diet 2. This data suggests a slightly better protein utilization of the formulations 1 and 8 over the commercial feed despite the slightly superior growth exhibited by the fish fed this diet. In other words, it seems possible that a slightly higher portion within the dietary protein of the commercial feed was partitioned towards the energy requirements and not for protein deposition in the fish carcass. It can be suggested, therefore, that there was no disadvantage from using the lower protein content (45% CP) adapted for the experimental practical diets or for that matter, in any of the previous experiments.

Due to unavailability of the same full fat soya meal as the one successfully used in the previous experiment, a full fat soya meal from a different source was included in the practical formulations tested in the present study. However, trypsin inhibition activity (TIA), cresol red values as well

as "available" lysine estimations did not differentiate this product with the full fat soya meal used previously.

The possible anomalies of these tests in relation to the inferior nutritional value of full fat soya bean meal when tested in practical diets for seabream are discussed later. The results reported in this section contrast with the performance of heat treated full fat soya in the previous study.

It is interesting that on examination of the data, a possible explanation for the reduced performance of seabream receiving diets with high full fat soya meal inclusions may also be related to the fiber content of the formulations. These diets exhibited a fibre content exceeding 5% of the feed composition and were noticeably higher compared to the rest of the practical formulations. Fibre is known to interfere with digestion rate in fish by promoting a faster intestinal transit time. It may also promote nutrient interaction and interfere with absorbance and utilization (Davies, 1985).

The digestible protein coefficients together with the digestible energy values calculated for the individual ingredients used in the present study do not, alone, explain the large differences in growth and feed utilization observed over the experimental period. However, the energy digestibility coefficient value for the specific full fat soya meal used was not determined. It is possible that a

different processing procedure can reduce the availability of the oil in the matrix of the material thus reducing the digestible energy content of the diets.

Values for the protein content of the diets were also expressed as digestible crude protein and although these were of similar value, data was not available for the individual amino acid availabilities. In addition, individual amino acids show some degree of competitive interaction, and it is possible that in multi ingredient formulations, amino acid antagonism may occur. The relationship between lysine and arginine, for example, has been well established in pig and poultry nutrition but not in fish (Wilson, 1985).

Palatability, although an important consideration when fish are fed to satiation, was not a significant factor in the present investigation. All fish were fed to a fixed feeding level of 4% of body weight per day and fed aggressively throughout the trials.

The carcass moisture and protein contents of the fish were found to be similar for all the groups fed the practical type diets at the end of the experiment. Seabream kept on control diet 1 and diet 8 (high fishmeal, high poultry meat meal) showed a significantly higher carcass lipid level ($p<0.05$) leading to greater fat deposition. This may be interpreted as an increased availability for dietary energy of these diets. The commercial feed and diet 3 (low fishmeal, high meat and bone meal) resulted in fish with a lower lipid content.

Carcass ash content was variable with the highest value of 5.21 found in the fish fed the commercial diet and the lowest value of 3.64 for diet 1 (all fishmeal control).

In conclusion, the practical diet evaluation for seabream confirmed the relative merits of several animal byproducts, especially poultry byproduct meal, as potential feed ingredients. The results for full fat soyabean meal, however, were quite discouraging compared to previous findings. Practical diet formulations are difficult to interpret due to their multi-ingredient nature. The interactions of different materials are complex and relate mainly to the variation in the source and processing technologies employed.

CHAPTER 6

6. GENERAL DISCUSSION

The theme of the current research programme was the evaluation of different animal and plant raw materials and byproducts in complete balanced diets for the gilthead seabream (Sparus aurata).

The classical fish nutrition experimental approach, where feed ingredient assessment and digestibility evaluation are combined in the experiment, was avoided in the present study.

A preliminary detailed first phase programme of this work was conducted to obtain the nutrient digestibility coefficients for some important classes of animal and plant derived products, and to provide a reference database for this species.

Following this first experimental phase, a range of raw materials was selected and tested at various inclusion levels in balanced diets for growth and feed utilization response by seabream. Selection was made according to the data obtained from the digestibility trial and also due to the potential market of these materials for the fish feed industry.

Finally, after establishing the most promising raw materials and the maximum possible incorporation in the formulation, a range of practical type diets were tested

using a least cost formulation approach.

The digestibility study was undertaken in the experimental rearing facilities of the National Center for Marine Research (NCMR) in Athens, Greece. The limited number of experimental tanks together with the nature of the experimental design resulted in a lack of sufficient replication of the tested diets. Relatively longer experimental trials were therefore adopted to emphasize any trends that might have developed. However, this cannot be considered as an alternative for replication since both biological rhythms as well as adaptive inducible enzymes of the gastrointestinal tract can alter the results over the experimental period (Pandian and Vivekanandan, 1985).

The temperature effect on the nutrient digestibility values obtained from this work was not determined. Research undertaken by many workers on the effect of temperature on the digestibility coefficients for fish is contradictory (Windel et al., 1978; Evans, 1989). The ability of fish to digest a diet is largely dependent on both the metabolic status and associated gastric evacuation rate (Smith, 1989). Both factors increase with temperature. Therefore, fish are able to absorb more of a nutrient from a given amount of feed at a higher temperature due to greater metabolic activity, but the time available for absorption will decrease due to higher gastric evacuation rate. This overall opposing effect depends on the relative rates of the two factors, which vary

with each nutrient and fish species. Temperature in the present study was held constant at 20^{o} C throughout the experimental period.

Salinity was also fairly constant throughout the duration of each trial, in the region of full strength sea water (36ppt). Reports evaluating the salinity effects on digestibility are limited. De Silva and Perera (1984), however, showed that salinity does not affect the digestibility efficiency of tilapia (Oreochromis niloticus).

Meal size was set at a fixed level of 2% of body weight daily during the experimental period. Jollivet et al. (1988) have reported an increase of digestibility efficiency of protein, lipids and starch with increasing meal size. Windell et al. (1978) and Andrews (1979), however, observed a change of digestibility of lipids and carbohydrates but not for protein. Feeding at libitum was avoided because it was thought that this would result in a depression of digestibility values due to an increased feed intake.

Particle size also affects the digestibility of feeds as reported by Gropp et al. (1979). Digestibility of dry matter, crude protein, ether extract and gross energy was significantly reduced by increased particle size. In this work all materials were ground to the same particle size of 0.2mm before compounding into a pelleted form.

Fish size is also reported to affect digestibility. Windell et al. (1978) showed that bigger fish exhibit higher

digestibility capabilities than smaller fish of the same species, probably due to an immature digestive system and lower enzyme activities. In the present study, the size of the fish was uniformly distributed among the experimental tanks. However, the size of the fish used for the digestibility trial was different from those used in the subsequent growth experiments. This was mainly done for practical reasons. These included experimental space limitations, amount of faecal material needed for collection and the cannibalistic character of younger fish.

Being a comparative study on the nutritional properties of the different materials tested, the relative differences of the performance obtained was of greater significance, and not their potential changes due to any biotic and abiotic effects.

Apparent and not true digestibility coefficients were determined in this work. The latter, would require the preparation of a zero protein diet fed to a group of fish, and hence the endogenous nitrogen losses would be assessed (Gropp and Tiews, 1981). However, major palatability problems associated with such a diet did not allow this approach to be followed.

Many researchers have reported the importance of using metabolizable energy (ME) and digestible energy (DE) values in diet formulation (Smith, 1979; Jobling, 1983). In this current work, only DE values were determined due to lack of

specialized equipment needed for measuring urinary losses to calculate ME. Furthermore, Jobling (1983) stated that ME is a unit which can never be accurately measured and it is suggested that dietary DE is a more realistic unit upon which to base nutrition and production studies.

In this study, all the energy estimations were determined using the chemical wet oxidation procedure using dichromate (O'Shea and Maguire, 1962). This method was used to overcome the problem associated with the small quantities of faecal material available for analysis. The method proved to be fairly fast and reliable, despite the small sample size used (50mg). Petihakis (1989) compared the energy values determined by bomb calorimetry, wet oxidation method and estimation of energy using proximate analysis and caloric conversion terms. His findings suggest that values obtained by bomb calorimetry and wet oxidation are in very close agreement, whereas energy estimated by the caloric conversion factors of nutrients is in most cases underestimated. Jobling (1983) recommends the use of bomb calorimetry as a direct and standard technique for gross energy determinations.

Another point of debate is the validity of protein content estimations by measuring the total nitrogen (N) content within the sample and converting this figure to a total crude protein value by using the conversion factor 6.25. This was the method employed in the present study and is the most commonly used procedure for protein estimation in

agricultural biochemistry. This method, however, employs two fairly unsound assumptions (Gropp and Tiews, 1981; Jobling, 1983). Firstly, that protein contains 16% nitrogen and secondly, that all nitrogen present in the material is bound in protein. Proteins vary in their amino acid composition and, therefore, the nitrogen content of proteins from various sources also tends to vary. Consequently a different conversion factor should ideally be used each time, depending on the source of the protein. Also, many nitrogenous, non protein compounds such as amides, glycosides and compound lipids may be present in the analyzed sample resulting in an overestimation of the protein content.

Finally, it must be mentioned that the digestibility coefficients of individual amino acids are the most efficient indication of protein quality and not the gross values present in the feedstuff (Ash, 1985). In the present work the determination of individual amino acid availability would have been beneficial. This, however, might be achievable when longer experimental periods can be employed and technical difficulties of faecal collection overcome.

In general the nutrient digestibility coefficients estimated here are in fair agreement with findings reported by many other workers.

The digestibility trials allowed a preliminary comparative assessment of several classes of feeding ingredients which appear to have potential use in practical feeds for the

seabream. Subsequent growth trials were necessary in order to confirm these initial findings.

A number of workers have tested new raw materials by either the partial or complete replacement of fishmeal in multi ingredient experimental diets resembling commercial diet formulations. This was the approach undertaken in growth trials for rainbow trout (Oncorhynchus mykiss) by Alexis et al. (1985) to evaluate poultry byproduct meal and plant protein sources such as corn gluten meal, soybean meal and carob seed meal. More recently, Davies and Wareham (1988) and Davies et al. (1990) employed a practical type basal diet in the evaluation of single cell protein and rapeseed meal respectively for the tilapia (Oreochromis mossambicus). Although these experiments offer useful information regarding novel feed ingredients, they are however, complicated with respect to the interpretation of such data. For example, a material of low nutritive value replacing fishmeal alone as the component of the diet might be negated prematurely if other, secondary protein sources were fixed into the formulation. A realistic optimum inclusion level for a material can only be allocated when comparisons are made against a high quality protein source such as fishmeal alone in diets for a given species. For these reasons, all the ingredients and byproducts were tested in semipurified diet formulations where a white fishmeal (Provimi-66) was the sole reference protein source. In this manner, maximum inclusion

levels for plant and animal byproducts could be obtained in the feeding trials undertaken in this study for seabream. Confirmation that this was a better approach is based on the recent work of Watanabe and Pongmaneerat (1991). These authors made a qualitative evaluation of some animal protein sources for rainbow trout which included white fishmeal, brown fishmeal, meat meal and meat and bone meal. The experimental design employed by these workers was actually a departure from the normal scheme of fish nutrition trials reported in the literature. Diets were formulated to contain different crude protein levels, in which each test ingredient was used as the sole protein source of the diet. By this manner, the relationship between the dietary protein level and biological value (BV) of the protein sources together with the optimum inclusion level could be assessed simultaneously within the same growth trial. In addition the latter workers were able to confirm that meat and bone meal was less efficiently utilized when compared to fishmeal. This was again attributed to some essential amino acids (lysine, methionine and tryptophan) being limiting in this product. The reduced performance of seabream receiving meat byproducts at high levels of inclusion was also discussed in this context. Watanabe and Pongmaneerat (1991) also stated that brown fishmeal was almost on par with white fishmeal with respect to its nutritional value. This finding has important implications to the choice of the white fishmeal in the

experiments reported here for seabream. It was observed that a commercial marine fish diet based on a herring type brown fish meal was only marginally superior in performance. This supported the further use of a white fishmeal reference diet in the subsequent experiments of this programme.

As pointed out in previous sections, poultry meat meals were better utilized by the sea bream than the meat and bone meals. This is supported by the findings of many researchers (Groop et al., 1979; Alexis et al., 1985) and is attributed possibly to the better amino acid profile exhibited by this range of products.

Turning our attention to the evaluation of plant products, soyabean meal and related materials were chosen to represent a non animal protein source. This choice was largely made with respect to the good digestibility profile obtained for seabream together with its role as the major alternative vegetable protein ingredient for other fish species (Dabrowska and Wojno, 1977; Jackson et al., 1982; Murai et al., 1989).

According to Akiyama (1988), plant protein feedstuffs are generally cheaper than animal protein feeds. Soyabeans and soyabean meals are increasingly utilized in fish feeds due to their good nutritional quality, lower cost and relatively stable world supply. The range of processing methods applicable to the raw bean are numerous and subject to much variation. Solvent extracted soyabean which has been

effectively heat treated is the most common form utilized in most feed mills. Preliminary investigations using this material for seabream diets followed a number of similar studies in other fish (Viola and Arieli, 1983; Aurelio et al., 1983). The results of this initial growth trial evaluation were used to select a threshold substitution level for fishmeal in a further assessment of related products differing only in processing technology. A spectrum of important parameters were used for the qualitative characterization of the selected materials originating from soyabean. These have been defined in an earlier section. It should be noted that these are subject to considerable variation due to the different methodology and laboratory standards employed throughout the world. In the present study, relative measurements played an important role in assessing the quality of the soy products tested. It should also be mentioned, that a common source of material was obtained for the full fat soyabean meal assessment, in which the material was heat treated under specified conditions in agreement with the manufacturers. Precise definitions are extremely important in any nutritional evaluation given the variation of ingredient composition and quality. As reported earlier, heat treatment in the form of toasting or extrusion cooking leads to an improvement in nutritional value for most non ruminant animals and fish by the thermal degradation of several antinutritional factors (Kaushik, 1989; Lovell,

1989). The results for seabream are in accordance with other findings for fish in general where effective heat treatment of soyabean meal led to an improvement in overall performance (Viola et al., 1983; Wilson and Poe, 1985a). Recently, Rumsey (1990) reviewed the importance of temperature in the commercial processing of soyabean meal products. Compared to the conditions reported for the study with sea bream in the current investigation, much higher processing temperature in excess of 200^{o} C led to an improved weight gain with rainbow trout. In a report for the American soyabean association, Vohra and Kratzer (1991) evaluated the adequacy of thermal treatment processes applicable to soyabean meal manufacture. These authors note the complexity of using a multiple index system for the in vitro chemical evaluation of such meals. It was stated that overheating rather than underheating is the most common problem encountered in quality control. Most parameters routinely used are too sensitive to quantify thermal damage and the consequently reduced availability of protein and essential amino acids. Given the recent developments in feed technology where high temperature expansion and extrusion systems are employed, better indicators will be required in the future.

The constraints for the inclusion of animal and plant byproducts obtained from the experiments reported with seabream led to the design of practical type diet

formulations. The results obtained from these later growth trials supported the importance of using reliable techniques for the qualitative assessment of individual ingredients. In the final trial, an apparently negative correlation was exhibited between growth and full fat soya meal inclusion. This finding contradicts the previous data when soyabean meals were tested as single ingredients. As mentioned before, the full fat soya meal used in the practical diets was from a different source than which formerly produced encouraging results. Although a number of tests were undertaken to assess the quality of this meal, which indicated a satisfactory quality, the outcome of the growth trial was disappointing. It is concluded that a wider spectrum of analytical methods and indices must be employed in order to fully assess the qualitative nature of these products. The findings also demonstrate the difficulty of applying data obtained from simple, single ingredient substitution studies in multi-ingredient formulations. It is apparent that a better knowledge of the interactions between nutrients and ingredients is essential in order to set realistic constraints on the choice of materials.

It is also important to note that for both the practical diet experiment as well as for all the other growth trials, the amino acid profile of each tested formulation was measured. These values, however, can only serve as a crude index of the quality of the protein. Individual essential

amino acid availability is the most solid criterion assessing the nutritional value of a given protein source (Wilson, 1985).

The linear formulation concept was employed to design the practical type feeds for the seabream. This system, however, has its drawbacks. One is that the system assumes a linear relationship between each of the ingredients used and each of the nutrient levels. However, this will not be valid in a number of cases. For example, the contribution to metabolizable energy of the first 10% of a feed ingredient in a diet may not be the same as the second (Crampton, 1985). In other words, the marginal utility of an ingredient for inclusion is not constant, though a linear programming model will assume that it is. A second assumption is that the parameters of interest can be stated in numerical terms. This can be difficult for parameters such as digestibility, since data are not present for all raw materials currently available. With other factors such as palatability, expression in numeric terms is almost impossible (Waldroup, 1984). The value of linear programming is greatly dependent on the quality and quantity of information about nutrient requirements and availability. However, the requirements for the nutrients by fish and the availability of these nutrients in different ingredients are not well known. Therefore, overemphasis on the least cost formulation of fish diets could be premature in light of the present state of knowledge

on fish nutrition (Cho et al., 1985).

Most growth trials with fish are performed using the juvenile stages of the species where the growth phase is rapid. Consequently, this was the main reason of using juvenile seabream for the sequence of growth trials described in this presentation. Constraints of both time and space within facilities resulted in a lack of suitable replication of some of the experiments. Restrictions with regard to the duration of the trials should also be considered. It must be emphasized that the results reported here are only outcomes of relatively short term experiments. Longer experimental periods or even growing the fish to marketable size would give a more complete picture of the nutritional value of the tested materials.

It is pertinent to perform growth trials with a homogeneous population of fish of known history and defined strain. All the investigations with gilthead sea bream reported in this programme of work were obtained from a common source and genetic pool and were therefore deemed to be comparative.

The growth trials were all undertaken in recirculation rearing units in order to keep the environmental conditions at desirable levels. It is well established that water quality parameters (temperature, salinity, ammonia, pH and dissolved oxygen) all affect growth performance (Brett, 1979). Great care was taken, therefore, to keep these

parameters constant at suitable levels reported in the literature for seabream.

Feeding ad libitum is recommended by many researchers (Gropp and Tiews, 1981; Jobling, 1983). According to these workers, it is very important that food availability does not act as a limiting factor for growth when conducting any nutritional study. A fixed feeding level was adopted for this present study. One reason was the fact that ad libitum feeding would exert high environmental loading to the recirculatory systems employed. Secondly, no palatability problems were observed in all feeding tests and the fish ate the whole daily ration aggressively. Thus the performance of the seabream relied mainly on the relative nutritional quality of the tested materials in relation to growth and feed utilization.

Earlier work on the nutritional requirements of the gilthead seabream (Sparus aurata) regarding the quantitative dietary protein and lipid levels were the basis for deciding the experimental dietary specifications (Sabaut and Luquet, 1983; Kissil, 1981).

Supportive evidence on the levels of major nutrients adopted come from Takeuchi et al., (1991) who established an optimum dietary protein and lipid content at 50 and 15% respectively in diets for red seabream Pagrus major.

It is of great significance to mention that all experimental diets were cold pelleted in the laboratory, with

temperatures never exceeding 40^{o} C during drying. These conditions are not by any means comparable to the conditions used in the feed manufacturing industry where temperatures often reach much higher levels for both standard steam pelleting and extruded type diets. These different processing conditions can alter the physical and nutritional properties of the feed, and hence the data obtained from the present study must be interpreted in this context. If the tested feeds were processed under different conditions, then the outcome could be different in future growth performance investigations.

The two most common processing methods for the preparation of dry pelleted diets involves the use of varying degrees of heat, moisture and pressure to produce expanded (extruded) pellets and standard pressure steam pellets (Hilton et al., 1981). Extruded pellets have advantages over steam pellets in that they generally have superior water stability and floating properties which allows direct determination of feed consumption (Stickney, 1979). However, much greater levels of heat, moisture and pressure are used in extrusion processing than in steam processing thus reducing protein and other nutrient availability (Lovell and Lim, 1978). Extrusion also promotes starch gelatinization in cereal ingredients and consequently improves carbohydrate digestion and absorption resulting in enlarged livers and increased liver glycogen content in carnivorous fish (Hilton et al.,

1981). Metabolic problems, possibly resulting from the overfeeding of diets containing elevated carbohydrates in winter diets for seabream, have been widely reported (Sweetman, pers. comm., 1991). This has been described as the "belly up" syndrome in Greece leading to significant mortalities in production size fish. Excessive visceral and hepatic lipid deposition has also been reported in bream at marketable weights.

Although most diets for marine fish are prepared conventionally, extrusion technology offers a possibility of incorporating more oil into the finished pellet, thereby allowing the manufacture of high energy feeds.

In experiments involving rainbow trout (Oncorhynchus mykiss), Roberts (1990) observed that energy dense, expanded diets were more efficiently utilized when supplied at similar rates compared to standard pelleted types. Recent environmental considerations have focused much attention on feeds capable of reducing pollution and new EEC legislation will soon be applicable to sea cage operations in the Mediterranean as well as Northern Europe. In this context, high energy protein diets fed at controlled levels offer the possibilities of greatly improved feed efficiency and reduced discharges from farms.

The findings presented in this study regarding the nutritional value and practical feeding levels of animal and plant byproducts for sea bream are an important consideration

in this aspect. Crampton (1987) outlined the possibility of formulating low phosphorus diets for use in aquaculture. Although soyabean meal has a relatively low phosphorus content, it is mainly in the form of phytic acid, and not biologically available. The same points also apply to wheat. This means that a low phosphorus feed can be achieved by including high levels of soya and wheat, but such a diet would produce poor feed efficiency and thereby high pollution levels (Ketola, 1985).

Meat and bone meals are characterized by their high ash contents and are particularly rich sources of calcium and phosphorus. The disadvantages of using such products in practical feed formulation for sea bream become obvious in view of these previous statements.

As a result of the recent outbreak of Bovine Spongiform Encephalopathy (BSE) in the United Kingdom affecting the dairy and beef industry, the raw materials available to the fish nutritionist became reduced. In Britain, pressure has been applied on feed manufacturers to remove substances that might be linked to such disorders, ie, blood and meat and bone meal (Springate, 1991).

Any removal of animal proteins as potential ingredients for fish food manufacture may only leave fish and vegetable products to provide the essential digestible protein for fish growth. Fortunately, such circumstances have not yet arisen in the Southern European countries producing marine fish

diets. Perhaps in the future, legislation governing health and hygiene in abattoirs and the associated rendering trade will enforce stricter control. A typical example is the Feeding Stuffs Regulation (1988) compiled by the UK Ministry of Agriculture, Fisheries and Food (MAFF) which defines quality criteria and specifications of raw materials for use in animal feeds. In particular, sterilization procedures to reduce the incidence of salmonella and other pathological agents will become increasingly important if animal byproducts are to have a wider acceptance in commercial fish feeds in Europe.

In conclusion, the research programme undertaken on the gilthead sea bream (Sparus aurata) has demonstrated the technical feasibility of evaluating a range of animal and plant byproducts using both digestibility and growth trial criteria. As far as possible, the data obtained for these ingredients was integrated to obtain a final assessment in terms of practical diet formulations for sea bream.

Clearly, animal byproducts such as poultry meat meal, poultry byproduct meal and meat and bone meal were feasible alternatives to the relatively expensive fishmeal component in test diets for seabream.

The original expectations for soyabean meal and related products were initially high, but later studies confirmed the variable aspects of quality and nutritional value as affected

by the degree of processing technology.

Future advances in biotechnology resulting in novel protein sources, and feed supplements such as purified amino acids will greatly influence our perception of applied fish nutrition. The addition of synthetic amino acids, for example, will lead to greater flexibility for the inclusion of raw materials in feed formulations for marine fish. The investigations presented in this research programme have only highlighted the potential value of selected raw materials and byproducts in complete diets for sea bream. Since mariculture is set to expand rapidly in the Mediterranean countries, the content and application of this research will be largely influenced by environmental, economic and political factors. It is these which control the National and International trade of feed commodities destined for aquaculture and which finally dictate the composition of feed formulations for fish.

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