STUDIES ON THE UTILIZATION OF DIETARY PROTEIN AND ENERGY BY GILTHEAD SEA BREAM (*Sparus aurata* L.)

A thesis presented for the degree of
Doctor of Philosophy to the University of Stirling

by

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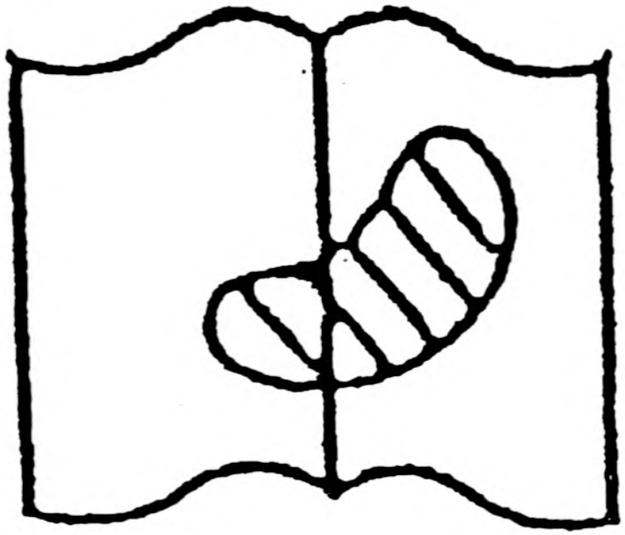

DECLARATION

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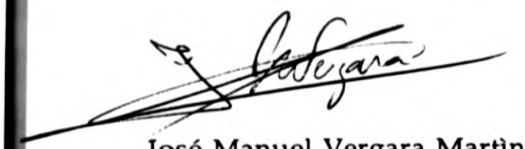

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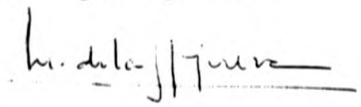

Dr. Manuel de la Higuera
Co-supervisor

ABSTRACT

A series of nutritional experiments were carried out to evaluate the utilization of dietary protein and energy by gilthead sea bream (*Sparus aurata* L.). Optimum dietary protein requirements and the sparing effect of dietary lipid upon protein were investigated for different fish sizes.

Optimum dietary protein requirements were 55% and 42% for 0.8g fry and 60g juveniles, respectively. In 5g fingerlings, dietary protein levels could be reduced from 52% to 45% when lipids were increased from 9% to 15%, best protein to energy ratio (P:E) being 21.9 g protein/MJ of gross energy. These results suggest that dietary protein level could also be reduced from 55% to 50% in fry diets containing 15% dietary lipids.

The optimum proportions of dietary protein and lipid level found for 90g growers were 54% and 11%, respectively, the high requirements for protein could be due to an increased protein demand during sexual maturation for gonad development.

The increase of dietary lipids produced an increment in carcass lipid deposition, both in visceral and non-visceral tissue, but these levels were in all cases well below reported carcass lipid contents in wild *S.aurata* in the Mediterranean.

When different dietary carbohydrate sources were evaluated with 42g *S.aurata* juveniles, the ability of fish to digest carbohydrate was limited in general, Apparent Digestibility Coefficient (ADC) values being lower than 85% regardless of carbohydrate source. Increased amounts of fibre in diets produced lower protein and lipid digestibility, this effect being even more pronounced on carbohydrate digestibility. Corn starch was the most effective carbohydrate source in terms of "energy-yielding", although wheat bran appeared to be a suitable fedstuff for practical diets.

Increased dietary lipid levels supported increased metabolizable energy (ME), recovered energy (RE), energy retention efficiency (ERE) and better protein conversion in 46g juveniles, indicating improved utilization of protein and energy.

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1. GENERAL INTRODUCTION

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1.1 Fish nutrition

Aquaculture has experienced its greatest development during the last 30 years. New species are being cultivated, new technologies have been introduced, a large research base has been established, and commercial investment is being directed into aquaculture. It presumably will supply an increasingly larger percentage of fishery products as time passes (Lovell, 1989).

The purpose of fish culture is to increase the weight of fish in the shortest possible time under economically acceptable conditions. A necessary condition is the optimal satisfaction of all physiological metabolic requirements of the organisms, as is attempted for example by the provision of favourable ambient conditions, and feeding with specially formulated diets (Steffens, 1989).

As culture technology has evolved, there has been a trend toward higher yields and faster growth. This has lead to enhancing the natural food available by fertilization (extensive systems), supplementing natural foods with moist or dry feed materials (semi-intensive systems), or supplying all the nutrients to the fish in a prepared diet (intensive systems). As the fish become more dependent on prepared feeds, the need for nutritionally complete feeds becomes more critical (Lovell, 1989; Tacon, 1990).

Research in fish nutrition has also developed significantly over the past 30 years, but the nutrition of terrestrial animals has been studied for a far longer time. Most of the early fish nutrition research was conducted with salmonid fishes. More recently attention has also been paid to other important species of fish cultured in different parts of the world, as well as new fish species with aquaculture potential (N.R.C., 1983; Steffens, 1989).

The nutritional quality of the diet is the foundation of fish farming, and can largely determine the success or failure of fish husbandry. Nutrition influences behaviour, structural integrity, general health, reproduction, environmental impact and growth in fish (Weatherley and Gill, 1987). Therefore, it is necessary to establish more precisely the nutritional requirements of fish under culture conditions so that well balanced diets can be formulated to maximize growth and also maintain the fish in good health

With the exception of water and energy, the dietary nutrient requirements of all aquaculture species can be considered under five different nutrient groups: proteins, lipids, carbohydrates, vitamins and minerals. The science of aquaculture nutrition is concerned with the supply of these dietary nutrients to cultured animals (Tacon, 1990)

Generally, fish are more efficient converters of feed to body weight than terrestrial animals, achieving whole body weight food conversion ratios of between 1:1 and 2:1, compared to 2.2:1 for chickens, 3:1 for pigs and 7:1 for beef

cattle and sheep (New, 1986). Conversion ratios on an edible flesh/food intake basis are even more favourable for fish, being about 2:1 compared to 5:1 for chickens and 20:1 for beef (New, 1986; Lovell, 1989).

When expressed as a percentage of the diet, fish require much higher levels of protein than do terrestrial farmed animals (Cowey and Sargent, 1979; Cowey and Luquet, 1983; Tacon and Cowey, 1985) and, since the protein component is the most expensive major ingredient in an animal feedstuff, some authors suggest that this requirement tends to lessen the advantages of fish as an efficient feed converter (Halver, 1976; Steffens, 1981). However, when dietary protein requirements are expressed in terms of protein intake rate (grams of protein per kilogram body weight per day) and the weight gain achieved per weight of protein ingested (grams of protein per kilogram live weight gain), the dietary protein requirements of fishes appear similar to those of terrestrial farm animals (Lovell, 1989).

In contrast to warm-blooded vertebrates, fish are poikilothermic animals and consequently do not have to expend a large proportion of energy in maintaining body temperature (Nijkamp *et al*, 1974). In addition, the primary end-product of nitrogen metabolism, ammonia, is rapidly excreted by passive diffusion through the gills, and consequently, fish employ less energy in protein catabolism than do terrestrial animals, which must convert ammonia to non-toxic substances such as urea or uric acid (Brett and Groves, 1979).

These two important metabolic differences between these vertebrate groups, according to Tacon and Cowey (1985), contribute to the high energetic efficiency of fish and thus, the absolute difference in requirements of fish and homeothermic vertebrates would reside in their requirement for energy, not protein (Cho and Kaushik, 1985).

Table 1 shows how the maintenance energy needs of fish are considerably lower than those of homeothermic animals.

Table 1. Maintenance energy expenditures in fish, chick and rat (Cho and Kaushik, 1985).

Species	Body weight (g)	Temperature (°C)	Energy ($KJ/Kg^{0.75}$.day)
Rainbow trout	50-150	20	33
Carp	40-90	23	54
Chicken	1500	Thermoneutral	355
Rat	130	22	552

When considering that up to the present time more than 300 different species of finfish have been cultivated, all with different feed and nutritional requirements, it becomes clear that much research work has to be carried out in order to achieve a basic knowledge of their nutrition (Watanabe, 1982).

1.2 Protein requirements

1.2.1 Protein metabolism

Proteins are complex organic substances composed of many amino acids linked together through peptide bonds and cross-linked between chains by sulfhydryl bonds, hydrogen bonds and van der Waals forces. In common with carbohydrates and lipids, they contain carbon, hydrogen and oxygen, but in addition also contain about 16% nitrogen (12-19% range), and sometimes phosphorus and sulphur (Tacon, 1990)

When proteins are hydrolysed with acid or alkaline solutions or enzymes, about twenty different amino acids are obtained, which are the end-products of protein digestion. The primary structure of proteins is polypeptides, the polymerised units of amino acids (Shepherd and Bromage, 1988).

Metabolism is the term given to the sequence, or succession, of chemical processes that take place in the living organism. Some of the processes involve the degradation of complex substances to simpler materials and are designated by the general term catabolism. Anabolism describes those metabolic processes in which complex compounds are synthesized from simpler substances. Waste products arise as a result of metabolism and these have to be chemically transformed and ultimately excreted; the reactions necessary for such transformations form part of the general metabolism. As a result of the various

metabolic processes energy is made available for mechanical work, and for chemical work such as the synthesis of carbohydrates, proteins and lipids (McDonald *et al.*, 1988).

The starting points of metabolism are the substances produced by the digestion of food. Digestion of proteins by fish enzymes such as pepsin, trypsin, chymotrypsin and carboxypeptidases, results in the production of free amino acids in the digestive system, which are then absorbed by passive and active diffusion via the intestinal villi into the blood stream (Halver, 1980; Weatherley and Gill, 1987). They are then carried to the liver where they may then be used for protein synthesis or may pass into the systemic blood and join the amino acids produced as a result of tissue catabolism in providing the raw material for synthesis of proteins and other biologically important nitrogen compounds. Amino acids in excess of this requirement are carried to the liver and broken down to ammonia and keto acids (carbon skeletons). The latter may be used for lipid synthesis (lipogenesis), carbohydrate synthesis (gluconeogenesis), amino acid synthesis or to produce energy (Weatherley and Gill, 1987; Hepher, 1988). The end-products of amino acid catabolism, ammonia principally, are eliminated by fish mainly through the gills (60-90%), the rest being excreted with urine, faeces and through skin (Walton, 1987).

Thus, the dynamic state of body protein is achieved through two processes: protein synthesis and protein breakdown. The difference between these two processes is used as a measure of "protein turnover". Mechanisms

involved in protein synthesis are similar in fish and in mammals, protein synthesis rates in fish being higher in liver, gill, digestive tract, kidney and spleen than in heart, red muscle and white muscle (Fauconneau, 1985)

The analysis of protein synthesis in whole fish shows that the overall quantity of protein synthesized by non-muscular tissues is more than twice as high as that synthesized by muscle which represents 40-60% of the body. Consequently, the greater part of dietary protein is used by fish to ensure protein turnover in non-muscular tissues (Fauconneau, 1985).

Fish muscle, on the other hand, carries out the main part of protein deposition at whole body level with a very low protein turnover compared to other tissues. This picture is also observed in mammals, but it is amplified in fish (Fauconneau, 1985).

Protein is the major organic material in most fish tissues, making up about 65 to 75 percent of the total on a dry weight basis and being, therefore, an essential nutrient (N.R.C., 1983; Hepher, 1988). Dietary protein is utilized by fish in three different ways (Cowey and Sargent, 1972):

(i) Maintenance (Synthesis of carbohydrates, lipids, proteins, hormones, enzymes, antibodies, and as energy source)

(ii) The repletion of depleted tissues

(iii) Growth (Synthesis of new additional protein tissues).

In animal production regimes, dietary amino acids are utilized for maintenance and growth. From a practical point of view, the ideal situation should tend to maximize the use of dietary protein for growth, minimizing the use of proteins for functional protein synthesis, gluconeogenesis, lipogenesis and energy (Higuera, 1987).

If adequate protein is not provided in the diet, there is a rapid reduction or cessation of growth or a loss of weight because the animal withdraws protein from some tissues to maintain the functions of more vital ones. On the other hand, if too much protein is supplied, proportionally less will be used to make new protein and the rest will be catabolized to produce energy (N.R.C., 1983).

1.2.2 Quantitative protein requirements

Growth rate is the most commonly used criteria to assess dietary protein level required by fish (De Long *et al.*, 1958; Hepher, 1988). The optimal protein requirement has been defined as the minimum amount of dietary protein, expressed as percentage of diet, needed to supply adequate amino acids and produce maximum growth (N.R.C., 1983); also as the protein level in the diet which tends to maximize simultaneously growth and protein deposition (Ogino, 1980; Weatherley and Gill, 1987). However, a considerable proportion of dietary amino acids is not used by fish for anabolic purposes, but is catabolized as an

energy source (Cowey 1979, 1980). Thus, Cowey and Sargent (1979) suggested that a better way of expressing the optimum dietary protein level is in terms of the proportion of energy it contributes (i.e., protein energy:total dietary energy). Similarly, Weatherley and Gill (1987) indicated that protein requirements should be expressed as suggested by the last authors or on the basis of weight gain (grams of ingested protein:Kg live weight gain).

The dietary protein requirements of fish were first investigated in Chinook salmon by De Long *et al.*(1958) and, since then, reported levels for various species range from about 30 to 55% (Table 2).

Many of the values in Table 2 are probably overestimated, and also are difficult to compare, due to one or more of the following reasons:

1) Most requirement values are based on levels of protein that result in maximum growth, with little or no index of protein utilization.

2) In many cases different protein levels were obtained by substitution of protein sources with carbohydrates such as dextrin or starches, and proteins, at least for salmonids, and probably for many other species, have higher metabolizable energy values than carbohydrates (Rumsey, 1978). Thus, fish fed higher protein levels may have used protein for energy purposes in a higher proportion than those fish fed the lower protein diets (Higuera, 1987).

Table 2. Estimated dietary protein requirement of selected fish species.

Species	Body wt. (g)	Crude protein level in diet for optimum growth (% dry wt.)	Reference
Common carp *Cyprinus carpio*	0.001-7	45	Sen *et al.* (1978)
	4-10	41	Takeuchi *et al.* (1979)
Grass carp *Ctenopharyngodon idella*	0.2	41-43	Dabrowski (1977)
Grey mullet *Mugil capito*	2.5	24	Papaparaskeva and Alexis (1986)
Tilapia *Oreochromis mossambicus*	2.0	40	Jauncey (1982)
Tilapia *Oreochromis aureus*	2.5-7.5	34-56	Winfree and Stickney (1981)
Tilapia *O.hornorum x O.mossambicus*	10.6	20	Clark *et al.* (1990)
Tilapia *Cichlasoma urophthalmus*	0.3	42	Martinez-Palacios (1987)
Blunt-snout bream *Megalobrama amplycephala*	4-37	27-40	Shi *et al.* (1988)
Major carp *Labeo rohita*	0.06-1.3	45	Sen *et al.* (1978)
Bighead carp *Aristichthys nobilis*	0.004	30	Santiago and Reyes (1991)
Channel catfish *Ictalurus punctatus*	200	32-36	Garling and Wilson (1976)
African catfish *Clarias gariepinus*	40-120	40	Machiels and Henken (1985)
Rainbow trout *Oncorhynchus mykiss* (*Salmo gairdneri*)	6.9	40	Satia (1974)
	6.5	50	Zeitoun *et al.* (1976)
Chinook salmon *Oncorhynchus tschawytscha*	1.5-5	40	De Long *et al.* (1958)
		44	Archdekin *et al.* (1988)
European eel *Anguilla anguilla*	7	45	Bilio *et al.* (1979)
	40	40	Higuera *et al.* (1989)
Japanese eel *Anguilla japonica*	2.5	44	Nose and Arai (1972)
Plaice *Pleuronectes platessa*	4-30	50	Cowey *et al.* (1972)
Gilthead sea bream *Sparus aurata*	3	40	Sabaut and Luquet (1973)
Red sea bream *Chrysophrys major*	16-45	54	Yone *et al.* (1974)
European sea bass *Dicentrarchus labrax*	5.5	40	Alliot *et al.* (1979)
Yellow tail *Seriola quinqueradiata*	65	55	Takeda *et al.* (1975)
Estuary grouper *Epinephelus salmoides*	60-70	40-50	Teng *et al.* (1978)

Table 2. (Cont.)

Species	Body wt. (g)	Crude protein level in diet for optimum growth (% dry wt.)	Reference	
Red drum *Sciaenops ocellatus*	4-5	44	Daniels and Robinson	(1986)
Puffer fish *Fugus rubripes*	2	50	Kanazawa *et al.*	(1980)
Smallmouth bass *Micropterus dolomieu*	0.6	45	Anderson *et al.*	(1981)
Largemouth bass *Micropterus salmoides*	2	40	Anderson *et al.*	(1981)
Halibut *Hippoglossus hippoglossus*	7	58	Hjertnes and Opstvedt	(1989)
Stripped bass *Morone saxatilis*	2.5 9-11	55 52	Millikin Berger and Halver	(1982) (1987)

3) Various investigators have used estimated gross energy values in formulating their diets (Cowey *at al.*, 1972; Teng *et al.*, 1978; Anderson *et al.*, 1981; Daniels and Robinson, 1986; Papaparaskeva and Alexis, 1986; Clark *et al.*, 1990); other authors used estimated metabolizable energy (Satia, 1974; Jauncey, 1982; Martinez-Palacios, 1987; Archdekin *et al.*, 1988; Higuera *et al.*, 1989); and also estimated digestible energy (Santiago and Reyes, 1991).

4) The protein sources may not contain adequate balance of essential amino acids.

5) A restricted or fixed feeding regime may have favoured the growth of those fish fed the higher protein diets, as fish eat to satisfy their energy requirements (Cowey and Sargent, 1979).

6) Most work was performed only with young fish.

7) Protein digestibility coefficients may vary as different protein sources are employed (Page and Andrews, 1973; Hunt, 1980; Tacon and Cowey, 1985 ; Wilson, 1985; Higuera, 1987).

1.2.3 Qualitative protein requirements

Much effort has been devoted to determine the qualitative amino acid requirements of fish, and for all the fish species so far investigated the same ten amino acids are essential: Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine. These amino acids cannot be synthesized "de novo" by the fish, and must be therefore administered in the diet (Steffens, 1989).

Most experiments used crystalline amino acids as the sole nitrogen source in the diets (Halver and Shanks, 1960; Nose *et al.*, 1974; Mazid *et al.*, 1978). Other experiments involved the injection of fish with radioactively labelled glucose (Cowey *et al.*, 1970).

The complete quantitative amino acid requirements have been established for only six fish species, namely chinook salmon, Japanese eel, carp, rainbow trout, tilapia and channel catfish (Jauncey and Ross, 1982; Lovell, 1989), and a limited number of requirement values have also been reported for the coho salmon, sockeye salmon, lake trout, gilthead sea bream and sea bass (Wilson, 1985).

1.2.4 Factors affecting protein requirements

The optimum level of protein in fish diets is influenced by fish species, protein quality (digestibility, available essential amino acid profile), dietary protein:energy ratio, the physiological state of the animal (size/age, reproductive state), environmental status (water temperature, salinity, etc.), and the level of food intake (Jauncey and Ross, 1982; Cho *et al.*, 1985).

Dietary protein requirements are said to be size-dependent, i.e., smaller fish require higher levels of protein for maximal growth than larger fish (Satia, 1974; Cho *et al.*, 1985). This effect is mostly associated with a large decrease in whole body protein synthesis throughout development in fish, leading to a decrease in growth rate in older fish (Fauconneau, 1985).

De Long *et al.*(1958) found that the optimum protein requirements of salmon increased significantly with increasing temperature. Similarly, Millikin (1983) found that increments in temperature increased the protein requirements of striped bass. Further contradictory results (Slinger *et al.*, 1977; Meade *et al.*, 1983; Goolish and Adelman, 1984) commenced a controversial debate and Cho *et al.*(1985) suggested that the protein requirements of fish are little influenced by temperature, as long as the fish are maintained within their normal temperature range.

Salinity can also affect dietary protein requirements in fish. Rainbow trout requires 45% protein at 20 ppt, and 40% protein at 10 ppt (Zeitoun *et al.*, 1974). Alliot and Pastoureaud (1979) suggested differences in osmotic regulatory mechanisms affecting protein requirements in fish, but there exist no data to clearly establish the effect of salinity on protein metabolism (Higuera, 1987).

Diminishing the feeding rate increases the optimal protein content in the diet for maximum protein retention in rainbow trout (Cho *et al.*, 1976). With tilapia, the maximum growth was achieved at a level of 25% dietary protein when the feeding rate was 3.5% body weight/day, while for a feeding rate of 2.9% the optimum dietary protein level was 30% (Wang *et al.*, 1985).

The influence of dietary protein to energy ratio and protein digestibility on protein requirements will be discussed in sections 1.3.3 and 1.4.2, respectively.

1.2.5 Protein sources

Until recently the tendency has been for commercial feed manufacturers to utilize high quality fish meals as the major portion of the protein sources in fish diets. The high cost of good quality fish meals leads to feed costs amounting to 40-60% of total operating costs in intensive aquaculture enterprises (F.A.O., 1983). Unfortunately, attempts by nutritionists to replace the fish meal component of practical fish feeds with alternative protein sources have met with

only variable success. Protein sources which have been considered include meat and bone meal, blood meal, soy bean meal, poultry by-products meal, dried brewers yeast, hydrolysed feather meal and corn gluten meal (Tacon and Jackson, 1985).

Apart from amino acid profiles that are often imbalanced, endogenous anti-nutritional factors and palatability or feed acceptability are among those factors limiting the use of these alternative protein sources, especially plant feedstuffs (Tacon and Jackson, 1985).

However, the increased availability of new processing technologies such as micronisation, extrusion and expansion, or the supplementation with limiting essential amino acids of alternative conventional feed ingredients, as well as the use of new, unconventional ingredients as protein sources (single cell proteins, plant protein concentrates, invertebrates and animal and food processing wastes), together with the use of dietary feeding stimulants or attractants, will, if not reduce the cost of the finished diet, at least allow it not to depend solely on fish meal. (Tacon and Jackson, 1985).

1.3 Protein and energy

Dietary protein is needed in order to provide fish with the adequate quality and quantity of essential amino acids to satisfy their requirements for

protein synthesis and other biologically important compounds. However, a considerable proportion of dietary amino acids is catabolized as an energy source (Cowey, 1979, 1980).

1.3.1 Dietary lipids as energy sources

Lipids normally occur in foodstuffs and in the fat deposits of most animals in the form of triglycerides, which are esters of fatty acids and glycerol (McDonald *et al.*, 1988).

The main products of lipid digestion in fish intestines are free fatty acids, most of which contain a single COOH group and a straight unbranched carbon (C) chain, which may in turn contain no double bond (saturated fatty acids), a single double bond (mono-unsaturated fatty acids), or more than one double bond (polyunsaturated fatty acids, PUFA). The latter may be divided into three major families: the oleic (18:1n9) series, the linoleic (18:2n6) series, and the linolenic (18:3n3) series (Tacon, 1990).

Most animals, including fish, are incapable of "de novo" synthesis of fatty acids of the linoleic series and linolenic series, so these fatty acids must be supplied within the diet (Tacon, 1990).

With the possible exception of carnivorous fish species, fish are able to chain elongate and further desaturate 18:2n6 or 18:3n3 to the corresponding highly unsaturated fatty acids (HUFA) 20:4n6 and 20:5n3 or 22:6n3, respectively (Watanabe, 1987). In general, cold, freshwater fish have an exclusive requirement for 18:3n3, 20:5n3 and 22:6n3 in their diet, while warm, freshwater fish have either a requirement for both linolenic series and linoleic series, or for the linolenic series alone. In the case of marine carnivorous fish species, since the food organisms consumed are rich in 22:6n3 and 20:5n3, it seems they have a very limited ability to chain elongate and further desaturate 18:3n3 to the corresponding HUFA. They must, therefore, be supplied with 22:6n3 or 22:5n3 in their diets (Kanazawa, 1985; Watanabe, 1987; Tacon, 1990).

Thus, dietary lipids provide a source of indispensable nutrients, the essential fatty acids. In addition, they also act as carriers of fat soluble vitamins (A, D, E and K) and they also are an important source of energy (New, 1986). Fats contain more energy per unit weight than any other biological compound.

This fact has been widely studied, and many experiments carried out with different fish species have shown that by increasing the dietary lipid content, the optimum level of dietary protein can be reduced due to the high effectivity of lipids as "energy-yielding" nutrients (See section 1.3.3). Increased dietary lipid levels have also been reported to increase metabolizable energy, recovered energy, energy retention efficiency and support better protein conversion, indicating improved energy utilization (Cho, 1987).

1.3.2 Dietary carbohydrates as energy sources

Carbohydrates are utilized by terrestrial mammals as an important source of energy. However, this does not seems to be the case in fish. Carbohydrate digestibility in fish varies according to its complexity, source, pre-treatment and level of inclusion in diets; there also exist differences between species (Walton, 1987).

The inability of fish to utilize dietary carbohydrate has been illustrated by glucose tolerance tests. Oral administration of glucose to different fish species led to a linear increase of blood glucose concentration, with poor response of plasma insulin levels. This implies that glucose levels in blood are poorly regulated by fish, their response being frequently similar to diabetic mammals (Palmer and Ryman, 1972; Bergot, 1979; Cowey and Walton, 1989).

The administration of glucose also produced a rapid accumulation of liver and muscle glycogen in fish (Cowey and Walton, 1989). Liver glycogen reserves are practically depleted after night starvation in mammals, while they are scarcely altered in fish even after several months of starvation (Chang and Idler, 1960; Dave *et al.*, 1975). It is probable that the energy demand of tissues such as brain and nervous tissue, which catabolize glucose, are met in fish by gluconeogenesis (from amino acids and lipids) rather than by glycogenolysis (Walton and Cowey, 1982)

Glycolysis is the major route of glucose catabolism in fish tissues. This series of reactions converts glucose to pyruvate with the generation of ATP.

It has been impossible to effect any change in hexokinase (one of the enzymes involved in glycolysis) activity by dietary means (i.e.: increasing dietary dextrin from 0 to 55%) in rainbow trout, indicating the inhability of this species to control blood glucose due to lack of glucose phosphorylating activity in the liver (Cowey and Walton, 1989).

On the other hand, glucose turnover in fish is only 1/10 to 1/20 that of omnivorous mammals, glucose utilization being lower in fish than in rats and other omnivorous birds and mammals (Cowey and Walton, 1989).

Other carbohydrates such as fibres, hemicelluloses, lignin and pentosans generally form indigestible fractions in the feed, often as pellet binders. The growth of some fish species tends to be depressed by the presence of about 8% of dietary fibre and is highly depressed when the fibre content reaches 20% (Schwarz and Kirchgessner, 1982; Hilton *et al.*, 1983; N.R.C., 1983; Bromley and Adkins, 1984; Wang *et al.*, 1985; Davies, 1985).

The fact that carbohydrates can substitute protein for growth in fish feeds has been reported for some fish species (Cowey *et al.*, 1975; Alliot *et al.*, 1979; Shimeno *et al.*, 1985). However, reports in the literature on the maximum levels

of dietary carbohydrates that can be tolerated by different species of fish often appear to be contradictory. This is probably due to the variable digestibility of the different carbohydrate sources used and the fact that not all studies used isoenergetic diets (Martinez-Palacios, 1987).

Carnivorous fish species are known to have a limited ability to use dietary carbohydrate, mainly due to a weak amylolytic activity in their digestive tract (Spanhof and Plantikow, 1983). Dietary carbohydrate levels beyond a certain point have been reported to depress protein digestibility, feed efficiency and growth, increase liver glycogen deposition, liver size, and even cause eventual mortality (Shimeno *et al.*, 1979; Kaushik *et al.*, 1989).

On the other hand, some species seem to have a certain ability to use dietary carbohydrate, with levels in the range 10-40%, promoting higher growth and better protein efficiency ratios than diets with no carbohydrates at all (Cowey *et al.*, 1972; Garling and Wilson, 1976; Shimeno *et al.*, 1979; Alliot *et al.*, 1979; Berger and Halver, 1987).

Thus, main sources of energy in fish seem to be proteins and lipids, carbohydrates being considered as uncertain and probably limited (Walton, 1987)

Typical values of heats of combustion (gross energy) for carbohydrate, protein and lipid are 17.2, 23.6 and 39.5 Kilojoules per gram, respectively (Brafield and Llewellyn, 1982; Jobling, 1983; Cho, 1987; Steffens, 1989).

1.3.3 Protein to energy ratio

The evidence of the use of dietary protein as an important energy source is being widely studied (Cowey, 1979, 1980), and it has been suggested that the high protein requirement is an artefact of the low energy requirement in fish (See section 1.1). In addition, a great percentage of ingested protein is quickly catabolized by fish, specially when an inappropriate balance of non-protein nutrients is present in the diet. (Cho *et al.*, 1985; Walton, 1987; Shepherd and Bromage, 1988).

As the use of protein as an energy source is wasteful from both the nutritional and economic points of view, it seems worthwhile to supply as much as possible of the required energy as carbohydrates and lipids rather than protein, and thus to lower the proportion of protein in the diet to the level needed for growth.

This reduction of the dietary protein requirement for maximum growth by increasing the level of dietary, non-protein, energy is termed "protein sparing effect" (Jauncey and Ross, 1982).

The percentage of the total dietary energy required from either protein or non-protein ingredients may be used to express the relationship between protein and energy in the diet, and very often the ratio of the dietary protein to

the dietary energy content is used to express the fact that the feed intake is primarily designed to cover the energy requirement, instead of the protein requirement, of the fish (Steffens, 1989).

The beneficial effects of the incorporation of protein-sparing nutrients have been widely studied and optimal ratios between protein and energy have been proposed for many species of fish. In Table 3 optimum protein to energy ratios (in grams of protein per Megajoule) in the diets for selected fish species are shown. These results are based on experiments where dietary lipid was the main source of non-protein energy and fish were fed diets containing a range of dietary protein levels (in all of them but in those from Yone *et al.*(1971), Cowey *et al.*(1975), Marais and Kissil (1979), Watanabe *et al.*(1979), Bromley (1980), where all diets contained a fixed dietary protein level), combined with a range of dietary lipid levels. In all the experiments shown in Table 3 the calorific value of the diets was calculated by using typical nutrient caloric contents, except in those by Marais and Kissil (1979), Bromley (1980) and Daniels and Robinson (1986), where calorimetric techniques were used. Digestible energy values were determined by digestibility trials in the work by Wang *et al.*(1985).

In some experiments carried out with rainbow trout reported in Table 3, dietary protein levels could be reduced to 35% with little effect on general growth performance by adding lipid to the experimental diets at 18% level

Table 3. Optimum protein:energy ratios (g protein/MJ) in the diets for different fishes.

GE=Gross energy; DE=Digestible energy; ME=Metabolizable energy, as the basis of calculation.

Species	Temperature (°C)	Body wt. (g)	P:E ratio (g prot./MJ)	Energy as the basis of calculation	Reference	
Tilapia *Oreochromis aureus*	31	2.5	29	DE	Winfree and Stickney	(1981)
	31	7.5	26	DE		
Tilapia *Oreochromis niloticus*	21-25	6	16-17	DE	Wang *et al.*	(1985)
Tilapia *O.niloticus x O.aureus*	23-26	1.6	18	ME	Shiau and Huang	(1990)
Channel catfish *Ictalurus punctatus*	27	200	21-28	GE	Garling and Wilson	(1976)
African ccatfish *Clarias gariepinus*	27.5	40-120	28	ME	Machiels and Henken	(1985)
Red drum *Sciaenops ocellatus*	22-26	4	21	GE	Daniels and Robinson	(1986)
	26-33	5	26-29	GE		
Rabbitfish *Siganus guttatus*	27-29	0.9	22	GE	Parazo	(1990)
Rainbow trout *Oncorhynchus mykiss*	12	4.8	19-24	GE	Lee and Putnam	(1973)
	12	40	22	GE	Higuera *et al.*	(1977)
	11	2	27	DE	Reinitz *et al.*	(1978)
	11-14	15	19	DE	Takeuchi *et al.*	(1978)
	14-18	15	18	DE	Watanabe *et al.*	(1979)
	7-15	20	22-23	DE	Cho	(1987)
Red sea bream *Chrysophrys major*	25	12	33	GE	Yone *et al.*	(1971)
Gilthead sea bream *Sparus aurata*	23-26	44	22	GE	Marais and Kissil	(1979)
	20-25	45-50	25	GE	Kissil and Groop	(1984)
	20-25	3	23	GE	Kissil and Groop	(1984)
European sea bass *Dicentrarchus labrax*	18	5	32	GE	Alliot *et al.*	(1979)
	19	75	29	GE	Metailler *et al.*	(1981)
Turbot *Scophthalmus maximus*	18	2-3	40	GE	Bromley	(1980)
Yellowtail *Seriola quinqueradiata*	29	65	26-35	ME	Takeda *et al.*	(1975)
	23	105	35	ME	Shimeno *et al.*	(1980)
	26-29	89	38	ME	Shimeno *et al.*	(1985)
Plaice *Pleuronectes platessa*	?	16-17	28	GE	Cowey *et al.*	(1975)
Striped bass *Morone saxatilis*	22	11-16	28	ME	Berger and Halver	(1987)
Walleye *Stizostedium vitreum*	21	14	34	ME	Barrows *et al.*	(1988)

(Takeuchi *et al.*, 1978, Watanabe *et al.*, 1979). All works listed showed some protein-sparing effect by dietary lipid.

Up to 1/3 of the dietary protein could be spared by lipids in turbot (Bromley, 1980), and dietary protein level could be reduced from 50 to 45% when lipid level was increased from 14 to 17% in striped bass (Berger and Halver, 1987).

A similar protein-sparing effect was reported for yellowtail (Takeda *et al.*, 1975; Shimeno *et al.*, 1980, 1985), although minimum optimum dietary protein level achieved ranged between 53 and 57%, given dietary lipid contents of 15-17%.

Hybrid *Oreochromis niloticus X O. aureus* showed satisfactory growth performance when the dietary protein content was decreased from 24 to 21% and dietary lipid raised from 9 to 15% (Shian and Huang, 1990). Channel catfish showed also a protein-sparing effect when a decrease from 36 to 24% in dietary protein gave similar performance when lipids were increased from 5 to 10% (Garling and Wilson, 1976).

In plaice and European sea bass, high lipid levels promoted a certain protein-sparing effect, but at the expense of an increasingly fatty carcass (Cowey *et al.*, 1975; Alliot *et al.*, 1979). Other authors report reduced growth

performances as a consequence of increased dietary lipid levels (Yone *et al.*, 1971; Machiels and Henken, 1985; Daniels and Robinson, 1986).

It appears from these data that there are considerable differences between species and, in general, the requirement of the fish for protein per unit energy intake (digestible or metabolizable) lies well above 20, and is greater than that of terrestrial animals (14-18 for chicken, 10-16 for swine and 6-10 grams of protein per Megajoule for cattle)(Cho and Kaushik, 1985).

On the other hand, the protein-sparing action of dietary lipid seems well documented, proving that in many cases the optimum level of dietary protein can be reduced at higher lipid inclusion rates. However, very often the level of lipid in the diet found necessary to maximize the protein available for growth may produce a high lipid carcass deposition (Cowey *et al.*, 1975; Alliot *et al.*, 1979), although this only occurs, according to Watanabe et al.(1979), when lipid is added to a diet already high in protein. A true protein-sparing effect could thus be achieved at somewhat reduced protein levels.

The optimum levels (i.e., those which support the best growth rate and food conversion ratio) of protein and lipid in a fish diet, depends on many factors such as the environmental conditions (temperature being probably the most important), fish age and size, genetic factors and the aquaculture system/techniques being used, among others (New, 1986).

The idea that dietary energy sources in fish diets influence the protein requirements of fish for optimum growth, and the metabolic partition of the dietary protein between use for protein synthesis and for energy supply is also supported by the results reported recently by Kim *et al.*(1991). They found that when rainbow trout fingerlings were fed different purified diets using casein as protein source and a mixture of crystalline non-essential amino acids as protein substitute, the optimum dietary protein level was found to be 24%, suggesting that this protein level was sufficient to provide the requirements of trout for essential amino acids. According to the authors, the conventionally established protein requirement of 40% for rainbow trout (Satia, 1974; N.R.C., 1981) includes dietary protein required to meet the requirements for the essential amino acids (24%) plus that required for meeting energy needs (16%). However, non essential amino acids are only interconverted, not synthesized *de novo* by fish, and they still will require some of the diet in the form of protein for this purpose (Jauncey, personal communication).

1.4 Nutrient digestibility

The main functions of the digestive tract in monogastric animals are the ingestion, digestion and absorption of food, and the elimination of solid waste material. Digestion processes basically involve the mechanical and chemical breakdown of food particles and their nutrients to provide the animal with the simpler forms of these nutrients, such as amino acids, fatty acids and glucose, required for metabolic purposes (McDonald *et al.*, 1988).

Fish vary tremendously in morphology and physiology of digestive tracts and in feeding behaviour. Among the most pronounced distinctions are that carnivorous fish have stomachs, while herbivorous fish, particularly cyprinids, frequently do not. Moreover, carnivorous fish species usually have digestive tracts less than one half the length of their body and herbivorous fish have tracts six to eight times their body lengths (Lovell, 1989).

In most carnivorous fish the digestion of food commences in the stomach. The gastric mucosa of the stomach secretes a protective mucus, plus pepsin, a protease. The glands of the stomach produce hydrochloric acid which maintain the pH of the stomach contents within the optimal range for pepsin activity (pH 2 to 4). Protein digestion proceeds in the intestine in an alkaline medium by the action of pancreatic trypsin and chymotrypsin. Trypsin is one of the most important proteases for protein digestion, and polypeptides are further broken down to peptides and free amino acids by intestinal peptidases and pancreatic carboxypeptidases (Weatherley and Gill, 1987).

Pancreas and liver are concerned with the production of those digestive enzymes, which include carbohydrases, which convert oligo and polysaccharides into simpler sugars and lipases to hydrolyse triglycerides (lipids) into diglycerides, monoglycerides, glycerol and free fatty acids (Shepherd and Bromage, 1988).

The dietary nutrients, broken down into simple compounds in the digestive tract, are absorbed through the intestinal epithelium by diffusion or by active transport. The waste material, or faeces, voided from the intestine via the anus consists mainly of water, undigested food residues, digestive secretions, epithelial cells from the tract, inorganic salts and bacteria (McDonald *et al.*, 1988).

1.4.1 Factors affecting digestion of food in fish

Digestion of food in fish depends on three main factors: a) The ingested food and the extent to which it is susceptible to the effects of the digestive enzymes, b) the activity of the digestive enzymes, c) the length of time the food is exposed to the action of the digestive enzymes. Each of these main factors is affected by a number of secondary factors, such as fish species (carnivorous versus omnivorous/herbivorous species), age, size and physiological condition; other factors are related to environmental conditions such as water temperature; and some are related to the food itself, i.e., its composition (the relative proportion of nutrients in the diet), particle size and amount eaten (Page and Andrews, 1973; Andrews, 1979; Hepher, 1988).

1.4.1.a Fish species

Nutrient digestibility may vary among fish species due to differences in the digestive system and its digestive enzymes, and to the different foods consumed. Despite these differences and the lack of pepsin in fish without stomach, variations in the digestibilities of proteins and lipids among species are small. Much more pronounced variations are found in digestibility of carbohydrates, specially those with high molecular weight (Hepher, 1988).

1.4.1.b Fish age

Enzymatic activity may vary with fish age and size, and usually is lower during first development stages of fish. This clearly affects digestibility of different nutrients (Hepher, 1988).

1.4.1.c Physiological conditions

Stressed fish, due either to excessive handling or to disease, may have a disturbed digestibility. Seasonal variations in digestive enzyme activity may also occur (Hepher, 1988).

1.4.1.d Water temperature

Increasing the temperature may increase both enzyme secretions and enzyme activity, and decrease enzyme-substrate affinity (Nordlie, 1966; Smit, 1967; Trofimova, 1973). Temperature may also affect the rate of absorption of digested nutrients through the intestinal wall (Smith, 1970; Smith and Kemp, 1971). However, the higher the temperature, the more rapid is the transport of food and the shorter its exposure time to the digestive enzymes (Elliot, 1972; Ross and Jauncey, 1981). Cho (1987) found no effect of water temperature variation on diet digestibility for rainbow trout.

1.4.1.e Water salinity

Not much is known of the effect of salinity or other factors related to water composition on digestibility. MacLeod (1978) found that the digestibility of dry matter, energy and protein by rainbow trout fell linearly with increasing water salinity, but this could be due to an indirect stress effect (Hepher, 1988).

1.4.1 f Food composition

The relative proportions of nutrients in the diet may affect their digestibilities, and increased amounts of dietary lipids are known to support increased protein, carbohydrate, lipid and energy digestibility (Takeuchi *et al.*,

1978; Watanabe *et al.*, 1979). Relatively high carbohydrate contents in the diet have been reported to reduce protein digestibility (Shimeno *et al.*, 1979; Kaushik *et al.*, 1989). The explanation seems to be that the undigested portion of the carbohydrates passes more rapidly through the alimentary canal, carrying with it some of the proteins (Hepher, 1988). Other carbohydrates such as fibres generally form indigestible fractions in the feed (N.R.C., 1983). Various feeds may also contain digestive enzyme inhibitors which reduce digestibility (N.R.C., 1983, Tacon, 1985). Food particle size is generally positively correlated with lower digestibility values (Hepher, 1988).

1.4.1.g Feeding level and frequency

Some authors suggest that some nutrient digestibility is negatively correlated with feeding level (Henken *et al.*, 1985). On the contrary, other works suggest that there is no direct effect (Windell *et al.*, 1978; Cho, 1987). Feeding frequency also does not seem to affect digestibility greatly (Hudon and De La Noue, 1984).

1.4.2 Protein digestibility

A distinction must be made between true and apparent digestibility of protein. The absorbed portion of a nutrient is usually determined by difference between the ingested and egested portions, and usually expressed as a

percentage of the amount ingested as "apparent digestibility coefficient" (ADC):

$$\text{ADC (\%)} = \frac{\text{nutrient ingested - nutrient egested}}{\text{nutrient ingested}} \times 100$$

In the case of proteins, there is a considerable nitrogen (N) fraction in the faeces resulting from maintenance metabolic processes, which is termed as "endogenous fecal nitrogen" (EFN), and can be quantified with the aid of a protein-free diet (Steffens, 1989):

$$\text{True N digestibility (\%)} = \frac{\text{ingested N-(Fecal N - EFN)}}{\text{ingested N}} \times 100$$

Another method for determination of protein digestibility is the "in vitro" method, which imitates the conditions in the digestive system of the fish as closely as possible, and consists of the incubation of protein-containing feeds with the digestive juices of the fish, followed by separation of the soluble products of digestion and quantitative amino acid determinations on the materials obtained (Grabner, 1985; Grabner and Hofer, 1985).

Although some authors have suggested that protein digestibility may vary with protein intake (Nose, 1967), fish size (i.e., small fish appear to be poorer at digesting protein than large ones), and water temperature (Kitamikado *et al.*, 1964), most works tend to show that most dietary proteins exhibit high true and, or, apparent digestibility coefficients (varying around 90%), and that

these digestibilities remain consistently high regardless of the level of protein fed (Kim, 1974; Cho *et al.*, 1976; Windell *et al.*, 1978; Austreng and Refstie, 1979; Jauncey, 1982).

On the other hand, food composition seems to affect more significantly the level of protein digestibility. Thus, increased amounts of dietary lipids have produced increased protein digestibility (Takeuchi *et al.*, 1978; Watanabe *et al.*, 1979).

In rainbow trout high levels of starch reduced the protein digestibility (Kitamikado *et al.*, 1964), and the higher the protein content and the lower the carbohydrate content are, the higher is apparent protein digestibility of compound feedstuffs. This seems to be true for most carnivorous fish (Shimeno *et al.*, 1979; Kaushik *et al.*, 1989) and some herbivorous fish (Page and Andrews, 1973). In carp the apparent protein digestibility was lowered by high cellulose content (18%) in the feed (Schwarz and Kirchgessner, 1982). This negative effect of indigestible crude fibre has been repeatedly reported for many fish species (Jobling, 1981; Hilton *et al.*, 1983; Wang *et al.*, 1985; Ferraris *et al.*, 1986).

Ash content also negatively affect protein digestibility (Nose and Mamiya, 1963).

1.4.3 Lipid digestibility

Digestibility of lipids range between 80-90% in most fish species (Cho *et al.*, 1982). Larger-chain fatty acids exhibit a higher digestibility than shorter-chain ones. Polyunsaturated fatty acids such as 20:5 or 22:6 acids are up to 100% digested by rainbow trout (Austreng *et al.*, 1980) and, in general, the essential PUFA show a very high digestibility in this fish species (Ellis and Smith, 1984).

Most of the time, this high digestibility of lipids is little affected by their inclusion levels in the diet or diet composition. Thus, for rainbow trout, lipid digestibility coefficients range between 93 and 98% (Higuera *et al.*, 1977; Takeuchi *et al.*, 1978). For European eel these values oscillate between 86 and 99% (Kuhne, 1973; Schmitz *et al.*, 1982), between 83 and 90% for carp (Takeuchi *et al.*, 1979; Kirchgessner *et al*, 1986), between 64 and 94% for channel catfish (Andrews *et al.*, 1978), and around 93% for tilapia (*O. niloticus*) (Hanley, 1987).

1.4.4 Carbohydrate digestibility

Considerable differences in carbohydrate digestibility between the various fish species can be expected as a consequence of the marked variations in the anatomy of the digestive tract and in the native diet (Steffens, 1989).

1.4.3 Lipid digestibility

Digestibility of lipids range between 80-90% in most fish species (Cho *et al.*, 1982). Larger-chain fatty acids exhibit a higher digestibility than shorter-chain ones. Polyunsaturated fatty acids such as 20:5 or 22:6 acids are up to 100% digested by rainbow trout (Austreng *et al.*, 1980) and, in general, the essential PUFA show a very high digestibility in this fish species (Ellis and Smith, 1984).

Most of the time, this high digestibility of lipids is little affected by their inclusion levels in the diet or diet composition. Thus, for rainbow trout, lipid digestibility coefficients range between 93 and 98% (Higuera *et al.*, 1977; Takeuchi *et al.*, 1978). For European eel these values oscillate between 86 and 99% (Kuhne, 1973; Schmitz *et al.*, 1982), between 83 and 90% for carp (Takeuchi *et al.*, 1979; Kirchgessner *et al*, 1986), between 64 and 94% for channel catfish (Andrews *et al.*, 1978), and around 93% for tilapia (*O. niloticus*) (Hanley, 1987).

1.4.4 Carbohydrate digestibility

Considerable differences in carbohydrate digestibility between the various fish species can be expected as a consequence of the marked variations in the anatomy of the digestive tract and in the native diet (Steffens, 1989).

A factor which has a major effect on carbohydrate digestibility in fish is the degree of polymerization. The monosaccharides are well absorbed by fish, while dextrin is only moderately digestible and crude starches have comparatively low digestibilities (Singh and Nose, 1967; N.R.C., 1983).

In addition, there may also be antinutrients found in natural feedstuffs which can inhibit the action of some hydrolases, as for example, the trypsin inhibitors present in many feedstuffs of plant origin, such as raw beans (N.R.C., 1983) or the amylase and protease inhibitors found in raw wheat (Tacon, 1985).

For salmonids the carbohydrate digestibility diminishes with increasing molecular weight (99% for glucose, 22% for polysaccharides)(Inaba *et al.*, 1963; Singh and Nose, 1967). The digestibility of polysaccharides is dependent on the magnitude of their contribution to the diet. Dextrin and starch are digested less well as their proportion in the diet increases, and cooked starch is significantly better digested than raw starch in rainbow trout (Buhler and Halver, 1961; Inaba *et al.*, 1963; Singh and Nose, 1967; Smith, 1971).

Similar results have also been reported for red sea bream (Furuichi and Yone, 1982), eels (Kuhne, 1973), plaice (Cowey and Sargent, 1972) and yellowtail (Shimeno *et al*, 1979).

Other carbohydrates, such as fibres, are considered indigestible fractions in the feed (N.R.C., 1983). However, a complete absence of crude fibre must be

regarded as unfavourable (Morita *et al.*, 1982; Furuichi *et al.*, 1983), as crude fibre delays absorption of carbohydrate and protein, and nutrients can be more effectively employed for energy production and biosynthesis. Fibre may also improve the pelletability of feeds (Jauncey and Ross, 1982).

The lack of apparent digestion or utilization of dietary fibre by most fish, despite many of them exhibiting some exogenous cellulase activity in their guts, has been interpreted as due to a considerable difference of the intestinal microflora of fish when compared to that present in mammals; low water temperature also negatively affects the microbial fermentation capacity of the intestine (Davies, 1985).

The commercially formulated diets used in fish culture result in the loss to the fish of between 20 an 40% of the intake in faeces. Thus, as a first step when evaluating potential feedstuffs, it is necessary to determine ingredient digestibilities prior to any attempt to formulate diets for fish (Cho, 1987).

1.4.5 Determining food and nutrient digestibility

Digestibility studies in fishes imply dealing with faeces collection in a aqueous environment. Nowadays, indirect methods are used based on the inclusion of inert marker in the experimental diets. Nose (1960) collected samples of rectal contents by "manually stripping" the fish and squeezing out

the fecal material from the rectum. Windell *et al.*(1978) utilized anal suction or fish dissection. Smith (1971) confined the fish in a metabolic chamber where faeces were collected as they were naturally produced by fish. Ogino *et al.*(1973) collected the faeces as the water outlet from fish tanks passed through a filtration column. Choubert *et al.*(1979) used a mechanically rotating screen to filter out fecal material.

Cho *et al.*(1982) developed a system consisting of passing the effluent water from the fish tanks through a settling column. The advantages of the last system are that it allows the fish to feed normally, there is no need to handle them, and it allows repeated determinations and evaluation of different diets by measuring growth rates and carcass analysis at the same time.

All of the above techniques have advantages and disadvantages; those based upon forced evacuation may result in the addition of physiological fluids and intestinal epithelium to the rectal contents, which may also not be completely digested, tending to underestimate nutrient digestibility. On the contrary, when faeces are diluted in water, leaching may result in overestimation of digestibility coefficients (Hepher, 1988).

Ever since Edin (1918) proposed the use of chromic oxide (Cr_2O_3), most digestibility trials in fishes have been carried out with this indicator. Relative changes in Cr_2O_3 percentage of the feed and faeces will represent the percentage

of the feed that was digested by the fish. By proximate and energy analysis of feed and faeces, the digestibility of each nutrient and the digestible energy of the diet can be determined (Cho, 1987).

Various other markers have been proposed for digestibility experiments with fish. Polyethylene and acid-insoluble ash have shown poor results in some cases, apparently because they move through the gastro-intestinal tract at a slower rate than the digesta, and show a wide range of variation in results (Tacon and Rodrigues, 1984). Crude fibre has also been proposed, based on the advantage of its similarity to the dietary material to be marked and good results obtained with its use (Tacon and Rodrigues, 1984).

Some doubts have been raised with regard to the suitability of chromic oxide as a dietary marker, due to its apparent differential passage along the gastro-intestinal tract with respect to the digesta. However, this variability in excretion pattern can be minimised by collecting faeces continuously over a period of days (Tacon and Rodrigues, 1984).

1.5 Energy utilization

Numerous authors have proposed schemes of the utilization and partitioning of food energy in animals, based on balanced energy equations that assign magnitudes to the various components of metabolism.

Kleiber (1961), Warren and Davis (1967), Elliott (1979) have all offered versions of such schemes with special reference to fish. Most recently reviews in energy partitioning in fish have also been published: Cho *et al.*(1982), Brafield (1985), Cho and Kaushik (1985), Knights (1985), Soofiani and Hawkins (1985).

Most of them depend on the essential assumptions of the type of energy equation given by Brett and Groves (1979), of which the authors point out that "since all the energy ingested (C) must turn up in one form or another as a result of metabolism (R), growth (P), nitrogenous excretion (U) and fecal loss (F)", therefore:

$$C = P + R + U + F$$

The expanded version of this equation is given also by Brett and Groves (1979) as:

$$C = (P_S + P_G) + (R_S + aR_{(R\text{-}S)} + bR_{(F\text{-}S)} + cR_{(A\text{-}S)}) + (U_U + U_S) + F$$

Where a, b and c are constants that apply to estimated periods each day for which routine, feeding and active metabolism are occurring, standard metabolism rate is R_S, routine metabolism is R_R, feeding metabolism is R_F and active metabolism is R_A.

P_S is somatic growth and P_G is gamete production. U_U corresponds to urea and ammonia excretion and U_S corresponds to mucus and sloughed epidermal cells.

There have been numerous attempts to compile and tabulate energy budgets for particular fish species: perch (Solomon and Brafield, 1972), brown trout (Elliot, 1976), tilapia (Mironova, 1976), rainbow trout (Staples and Nomura, 1976), sockeye salmon (Brett, 1983). Brett and Groves (1979) arrived at the following generalized budgets for young fish (figures are percentages of food energy, C):

Carnivorous: 100C = 29P + 44R + 7U + 20F

Herbivorous: 100C = 20P + 37R + 2U + 41F

Cho (1981) proposed a similar scheme of energy utilization in fish (Figure 1), which will be used here to describe the various physiological processes involved.

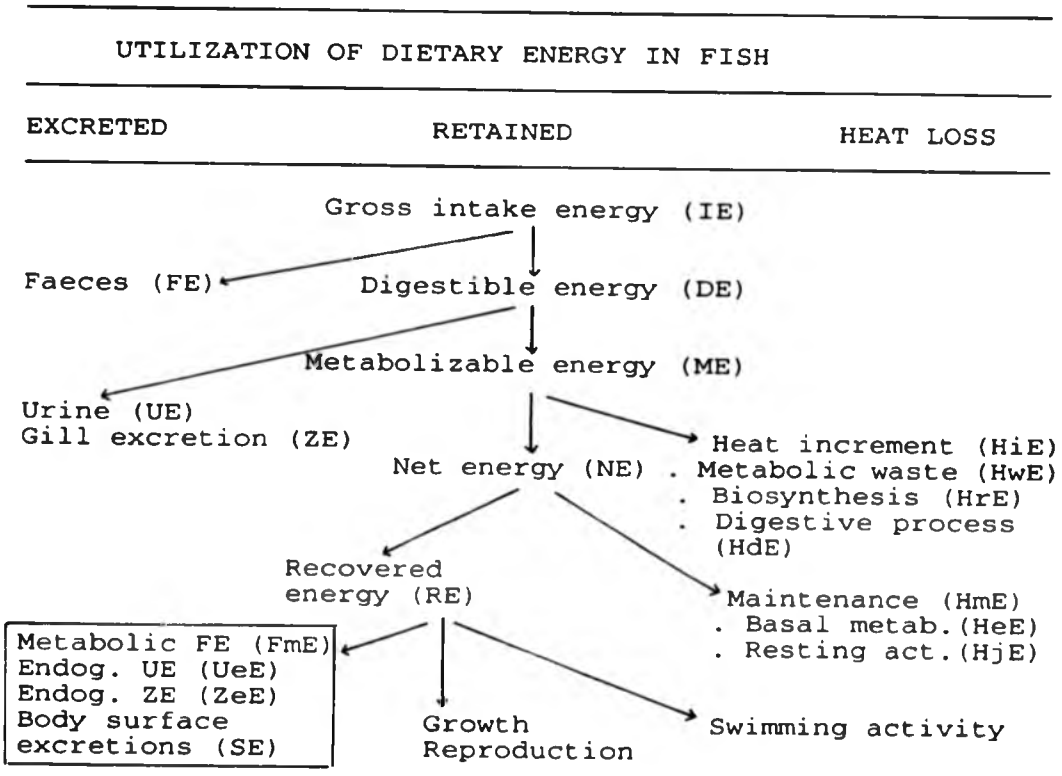


Figure 1. Utilization of dietary energy in fish
Modified from Cho (1981)

The gross energy intake (IE) is the product of ingested food and its heat of combustion. The first source of loss to be considered is that of the energy contained in the faeces. The apparently digestible energy (DE) of a food is the gross energy intake less the energy contained in the faeces (FE), which results from any particular input of that food (McDonald *et al.*, 1988).

Faeces are a mixture of undigested food and residues of body origin resulting from mucosa epithelial cells, gastrointestinal secretions and gastrointestinal flora activity. The energy loss due to these residues arising from metabolic activities of the animal is termed as fecal energy of metabolic origin (FmE). Similarly, other endogenous waste materials from metabolic activities will be excreted either through the gills (ZeE) or through the kidney (UeE). Mucus and epidermal cells will also represent an energy loss (SE) through the body surface (Cho *et al*, 1982).

The end-products of absorbed fatty acid and sugar catabolism are carbon dioxide and water. In addition to these products, catabolism of amino acids also results in ammonia production, together with minor quantities of other substances such as urea, uric acid, creatine, etc. The excretion of such products implies the loss of combustible material by the fish. Thus, the energy loss with these compounds either through the gills (ZE) or through the urine (UE) will decrease the physiologically available fuel value of the diet, the metabolizable energy (ME), defined as follows:

$$ME = IE - (FE + UE + ZE) \text{ (Fig. 1)}$$

The expenditure of energy due to feeding is referred to by several terms: heat increment of feeding (HiE), specific dynamic action (SDA), calorigenic effect and dietary thermogenesis. The main factors involved in this heat increment due to feeding activity are: 1) the digestion and absorption processes (HdE), 2)

biosynthesis of substances and their retention in tissues (HrE) and 3) production and excretion of metabolic wastes (HwE). The main biochemical cause for heat increment being the energy required for the ingested amino-nitrogen to be deaminated and excreted (HwE) and metabolic work (HrE) (Cho *et al.*, 1982).

The deduction of heat increment of feeding from the metabolizable energy gives the net energy value (NE) of the food, which is available to the animal for body maintenance and for the various forms of production (McDonald *et al.*, 1988).

Fish require a continuous supply of energy for maintaining life regardless of whether or not it is consuming food (HmE). Fasting fish obtain this energy by catabolizing body fat and protein reserves. A major portion of maintenance energy required is spent in basal metabolism (HeE) and a minor part is spent in involuntary or resting activity (HjE) (Cho *et al.*, 1982).

Any activity results in an increase in the basal metabolism rate. As stated previously, the ingestion of food increases metabolic rate. Physical activity (swimming) also increases metabolic rate due to work done against internal and external frictional forces. Thus, HmE, Hie and swimming activity lead to the release of energy as heat (HE) from the metabolizable energy derived from food, and this lost energy is not available to be retained within the body as new tissue elements (growth and formation of sexual tissues and products) (RE). In growing animals part of the retained energy is stored as protein, part as fat and

part as carbohydrate (glycogen)(Cho *et al.*, 1982), and the amount of energy so used is referred to as the animal's energy retention (McDonald *et al.*, 1988). The relative importance of different nutrients deposition depends upon a great number of factors, the balance of the available amino acids of the dietary protein and the amount by which the dietary energy intake exceeds the energy expended as heat being the two major factors (Cho *et al.*, 1982).

1.5.1 Factors affecting energy utilization

Tacon (1990) lists a series of factors known to influence the energy requirements and energy partitioning of fish, including:

(i) Water temperature (affecting metabolic rate, and consequently maintenance energy requirements, increasing with temperature).

(ii) Animal size (affecting metabolic rate, and consequently maintenance energy requirements, decreasing with increasing animal size).

(iii) Physiological status (increasing energy requirements during periods of gonad production and reproductive activity such as spawning migration).

(iv) Water flow (increasing energy requirements for maintaining station in water with increased water flow).

(v) Light exposure (energy requirements for voluntary activity being lower during night time "rest" periods).

(vi) Water quality and stress (pollutants, increased salinity, low dissolved oxygen concentration, and excessive crowding increasing the maintenance energy requirements).

Other factors which have also been reported are the relative proportion of dietary nutrients and feeding rate (Cho *et al.*, 1976).

Different authors have studied the effect of several factors on the energy retention by fish (for comparison purposes, energy retention will be expresed as energy gain/energy intake X 100 as a measure of energy utilization) (Steffens, 1980).

Cho and Slinger (1980) reported that falling water temperature in the range from 15 to 7.5°C increased the heat production in rainbow trout from 11 to 20% of the intake of digestible energy, hence lowering the energy retention from 58 to 44% of digestible energy intake.

Protein intake influences particularly the thermal gain. Increased protein contents in the diet (from 36 to 47%) lead to greater heat production (from 7 to 13% of DE) in rainbow trout (Cho, 1982).

The relative proportion of non-protein energy in the diet is another major factor influencing energy retention. At 36% of dietary protein an increase in the proportion of lipid from 6 to 16% lead to a reduction in the rate of heat production from 14 to 7% of DE, which was manifested in improved energy retention in rainbow trout (Cho, 1982). In the same species, the level of dietary energy retention was 53% of DE for a high-protein diet (55% digestible protein, 13% digestible lipid) and 63% for a low protein diet (34% digestible protein plus 22% digestible lipid) (Cho and Kaushik, 1985).

In carp, the value of dietary energy retention was 35% of digestible energy for a high protein diet (41% crude protein, 16.9 MJ/Kg diet of digestible energy) and 53% for a low protein diet (20% crude protein, 18.2 MJ/Kg diet of digestible energy) (Kirchgessner *et al.*, 1984).

In European eel, the level of dietary energy retention was 50% of gross energy for a high-protein diet (50% crude protein plus 20% wheat meal) and 67% of gross energy for a low-protein diet (20% crude protein plus 56% wheat meal) (Degani and Viola, 1987).

These examples underline how the understanding of the nutritional energetics of any productive animal helps the definition and balance of dietary regimes, under any particular environment, to improve the efficiency of diet utilization (Cho, 1987).

1.5.2 Determining energy utilization

Making estimations of intake gross energy and fecal energy is not difficult, for one only needs to know the energy content of representative samples of the food and the faeces (Brafield, 1985). On the contrary, measurements of metabolizable energy and heat losses in fish are far more complicated, as they involve the use of complex techniques which also lead to inaccuracy due to alterations in the normal fish metabolism through stressful handling of the animals (Cho, 1987).

There are various techniques available for measuring total ammonia in aqueous solution which have been used to estimate non-fecal nitrogen excretion in fish, including enzymatic assays (Cameron and Heisler, 1983), ammonia electrodes (Kaushik, 1980), chemical titration, and even indirect methods based on oxygen consumption (Brafield and Solomon, 1972).

Smith *et al.*(1978) designed a direct calorimeter to be applied in fish studies, but a most widely used technique is indirect calorimetry based upon the measurement of oxygen consumption (Solomon and Brafield, 1972; Cho *et al.*, 1975; Brett and Groves, 1979).

However, there is a simple schedule, developed by Cho and Kaushik (1985) involving the estimation of digestible energy and comparative carcass analysis to determine metabolizable energy and recovered energy. Digestible energy is easy to determine and fish are more suitable for comparative carcass analysis than other animals because it is possible to sample and homogenize a large number of fish with relative ease.

This schedule is summarized in Table 4, and was used with *Sparus aurata* juveniles, as part of the present work.

Table 4. Fate of dietary energy in fish through digestibility analysis and comparative carcass analysis (Cho and Kaushik, 1985).

---

1) Measure of digestible nitrogen intake (DN) and digestible energy (DE) intake.

2) Measure of recovered nitrogen (RN) and energy gains (RE) in fish carcass.

3) Estimation of non-fecal nitrogen losses:

$$ZN + UN = DN - RN$$
$$ZE + UE = 24.9\ KJ/g\ N \times (ZN + UN)$$
$$ME = DE - (ZE + UE)$$
$$HE = ME - RE$$
$$ERE = RE/DE$$

Where:

ZN = branchial N loss
UN = urinary N loss
DN = digestible N intake
RN = recovered tissue N
ZE = branchial energy loss
UE = urinary energy loss
ME = metabolizable energy
DE = digestible energy
RE = recovered energy
HE = total heat loss
ERE = energy retention efficiency

1.6 The gilthead sea bream

The gilthead sea bream (*Sparus aurata L.*) (Superorder: *Teleostea*, Order: *Perciforme*, Family: *Sparidae*) (Figure 2), is a marine, temperate water fish species. Its habitats are rocky and sandy bottoms of coastal water and river deltas, where it thrives in groups of year class individuals (F.A.O., 1976).

Juvenile and adult animals migrate to coastal brackish lagoons during the spring season, where they stay until October-November, when they return back to open coastal waters for spawning, usually in deep waters (Suau and Lopez, 1976). The spawning season in the Mediterranean region covers October to February (Coll, 1983). It is a eurythermal (10-36°C) and euryhaline species (salinity tolerance range: 5-60 ppt) (Coll, 1983, Chervinski, 1984). Abundant in the Mediterranean sea, it has also been reported in the Black sea and East Atlantic coasts, from Great Britain to Senegal (F.A.O., 1976).

Considered as a carnivorous species, its natural diet is composed mainly of crustaceans, molluscs, polychaetes, echinoderms and teleosts, prey size being positively correlated to fish size (Suau and Lopez, 1975; Arias, 1980; Ferrari and Chieregato, 1981; Wassef and Eisawy, 1985).

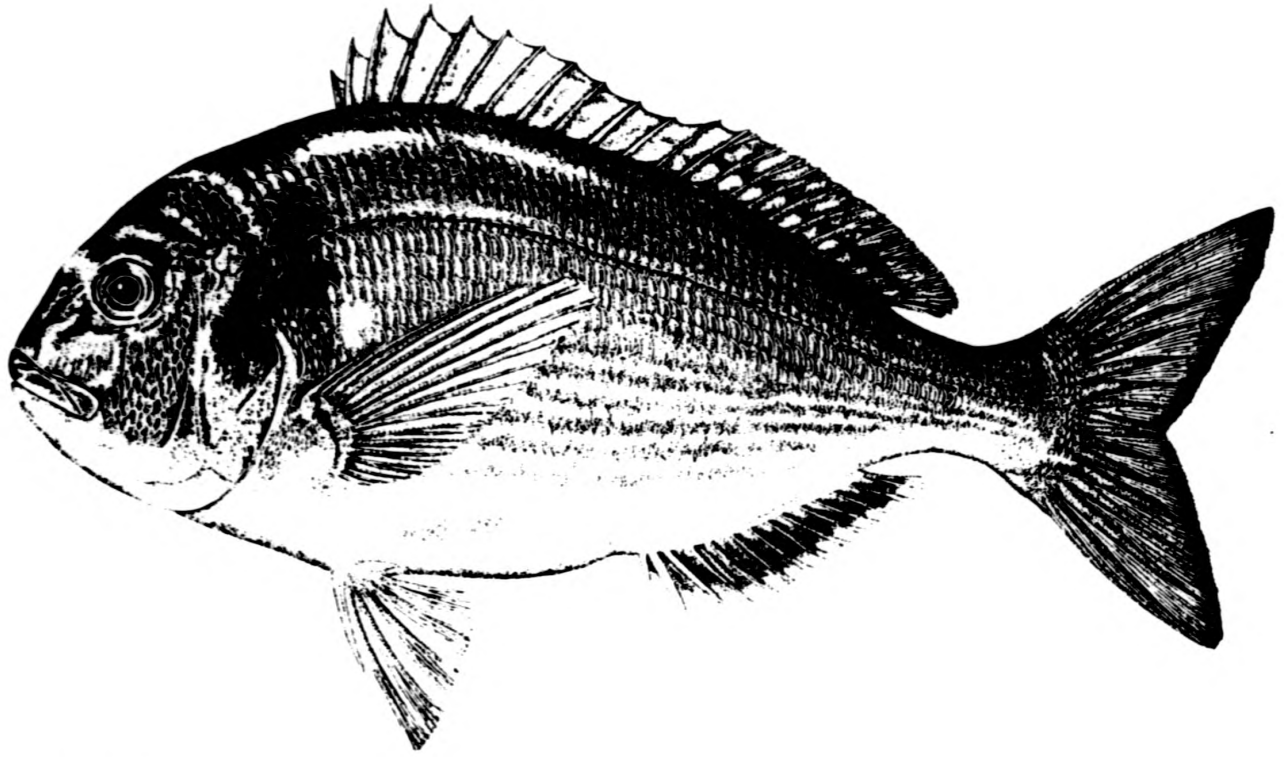


Figure 2. The gilthead sea bream (Sparus aurata). Pedro Salgado. 1990

Fisheries landings of this species have been sustained for the time period 1982-1988 at about 4,500 metric tonnes per year, from the Mediterranean area alone (F.A.O., 1990).

Sparus aurata was first cultured in the early 1970's in extensive systems in Italy ("valli-culture") (Faranda, 1977). This consisted of stocking wild juveniles into coastal brackish lagoons, often in polyculture, and harvesting them every 2-3 years with a size of 400g. High market prices and ease of acclimation to rearing conditions soon made the seabream a species much in demand from farmers who believed in its potential as a source of income even under intensive culture conditions.

Since then, major progress has been made in pond culture, which is still under development (Popper and Zoar, 1988). Improved management is being achieved by controlling fish migration and adjusting fishing techniques in the coastal lagoons (Chauvet, 1984). In addition, the expansion of existing areas in production, including countries mainly in the Mediterranean region (Gordin, 1983; Ledoux, 1983; Arias *et al.*, 1984; Chauvet, 1984; Eisawy and Wassef, 1984; Ravagnan, 1984; Barbaro *et al.*, 1986; Popper and Zohar, 1988), has lead to a major constraint: the decreased availability of fry from the natural environment (Ravagnan, 1984).

The above limitation encouraged a significant research effort into production of fry under controlled conditions. Thus, environmental and

hormonal control of reproduction, larval and fry rearing, live food production for larval stages and hatchery technology became major areas of research (Barnabe, 1976; Suau and Lopez, 1976; Arias, 1977; Coll, 1983; Barnabe and Billard, 1984; Kadmon *et al.*, 1985; Popper and Zoar, 1988). As a result, the "state of the art" in *S. aurata* hatchery production is now, although still in a transitory period, at a level where commercial hatcheries are currently operating (Lisac, 1990).

Intensive culture systems are also proving to be appropriate for this species when site characteristics do not allow for extensive or semi-intensive operations. This trend towards intensification is being mainly directed into floating cage on-growing systems (Pitt *et al.*, 1977; Porter, 1981), and integrated intensive farms including hatchery and cage on-growing facilities are nowadays operating on a commercial basis (Frentzos and Sweetman, 1989), as well as land-based on-growing farms.

During the last five years the farmed production of marine fish, such as European sea bass (*Dicentrarchus labrax*) and gilthead sea bream in the Mediterranean region has been characterized by its rapid growth (Ricard, 1990). In 1987 farmed output was in the region of 1,500 metric tonnes (of which 300 mt were sea bream) (Ricard, 1990). In 1989, estimated hatchery production of fry of both species totalled 29 million, twice the output of 1987 (Pauw and Billard, 1990). The yield forecast for 1991 is of some 2,727 tonnes for sea bream and 2,036 tonnes for sea bass (Flos *et al.*, 1990). Unfortunately, no data is available

on the relative contribution of intensive production to the above figures, and extensive, semi-intensive and intensive production must therefore be considered together.

According to Frentzos (1990), the unit production cost of sea bream in on-growing operations can nowadays be broadly broken down as follows: 40% fry production, 30% feed input, and 30% other costs. F.A.O. (1983) reports that feed costs amount to 40-60% of total operating costs in intensive aquaculture enterprises. With the present trend towards an increase in hatchery technique development it is likely that a significant decrease in fry production costs will make farmers and fish feed producers concentrate on feed quality and cost-effectiveness.

At present, conversion rates on sea bream farms are in the region of 2.5:1 or worse, while in the salmon farming industry conversion rates are well below 2:1 (Frentzos, 1990). Many commercial diets presently available for this species basically consist of modified salmonid diets, their nutritional efficiency being tested by trial and error (Apoyo Tecnico a la Piscicultura - ATP, A.S. Personal communication).

1.6.1 Gilthead sea bream nutrition

Initial research in gilthead sea bream nutrition were mainly carried out in France and Israel (Sabaut and Luquet, 1973; Luquet and Sabaut, 1974; Pitt *et*

al., 1977; Marais and Kissil, 1979; Kissil et al., 1981). Since then, an increasing amount of work has been done covering several aspects involved in the nutritional requirements of this species. The anatomy and histology of the digestive tract, as well as the activity of some enzymes involved in digestive and metabolic processes (glucagon, phosphatases) have been studied (Gutierrez *et al.*, 1985, 1986; Cataldi *et al.*, 1987).

Luquet and Sabaut (1974) determined the quantitative requirements of four essential amino acids in feeds for this species. Reported values, as a percentage of dietary protein, were: Arginine, < 2.6; Lysine, 5.0; Methionine + Cystine, 4.0; Tryptophan, 3.0.

Kissil and Koven (1984) tested semi-purified and purified diets with this species and found that amino acid supplementation of a casein-based diet resulting in a balance similar to hen's eggs protein, promoted satisfactory growth.

Up to date, the only work reported on quantitative protein requirement of the gilthead sea bream found a minimum dietary protein level for optimum growth of 40% for 3g initial body weight fish (Sabaut and Luquet, 1973). Fish were fed six semi-synthetic diets for 4 months containing protein in a range from 11.5 to 60.8% on a dry weight basis. Casein supplemented with a mixture of synthetic amino acids in order to obtain a balance similar to hen's eggs protein, was used as the main protein source. Dietary lipid levels were

maintained at 8%, consisting of a mixture of soy bean oil (5.7%) and cod liver oil (2.3%). The protein content in the diets was adjusted by substituting the nitrogen mixture with raw corn starch on a dry weight basis.

Koven and Kissil (1984) report that the highly unsaturated fatty acids (HUFA) 20:5n3 and 22:6n3 were required by *S. aurata* fry, but they were not in the position to determine whether gilthead sea bream has the ability to synthesize, elongate and desaturate the linolenic, 18:3n3 series precursors. In a similar sparid species, the red sea bream (*Chrysophrys major*), Kanazawa *et al.* (1979) and Yamada *et al.* (1980) found that although there seems to be a metabolic pathway for linolenic acid (18:3n3) to higher unsaturated fatty acids, conversion is slow and may be insufficient to meet the requirements of the essential 20:5n3 and 22:6n3.

Marais and Kissil (1979) found that no improvement in growth was obtained in *S. aurata* juveniles beyond 8-9% dietary lipids, when soy bean oil was used as main source of lipids.

Kissil and Gropp (1984) suggest that 10% and 5% dietary lipid contents are optimum for 3g and 45g *S. aurata*, respectively, and that capelin oil gives better results than soy bean oil as a dietary lipid source for this species.

Furuichi and Yone (1981) suggest that red sea bream is naturally potentially diabetic, and that long term feeding of high carbohydrate diets may

reduce the utilization of dietary carbohydrate. The lowest plasma insulin levels were produced in those fish being fed on the highest (40%) dietary dextrin level. Furuichi and Yone (1982) found that the availabilities of glucose and dextrin in red sea bream diets were considerably inferior to that of α-starch. In this species, dietary glucose levels above 10% depressed weight gain, protein conversion efficiency, increased liver size and promoted significant liver glycogen deposition (Furuichi *et al.*, 1971).

In *S. aurata*, Mazzola and Rallo (1981) successfully used a feed with a carbohydrate level of 31% on a dry weight basis, with wheat meal as main carbohydrate source.

Different commercial diets fed to *S. aurata* in France and Italy with carbohydrate levels varying from 14 to 29%, contained wheat meal and pregelatinized wheat starch as main sources of carbohydrate (New, 1986).

With respect to protein to energy ratio, Marais and Kissil (1979) reported that low energy diets (P:E ratio = 22mg/KJ) supported better growth performance of 44g *S. aurata* juveniles than high energy diets (P:E ratio = 20mg/KJ). Fish were fed for three months, diets containing a mixture of fish meal, meat meal and soya meal as protein sources, and soy bean oil as main source of lipids. Different P:E ratios were primarily achieved by ranged levels of protein (41-44%) and lipid contents (9-16%) in the diets.

Kissil and Gropp (1984) found that best P:E ratio for 45g *S. aurata* juveniles was 25 mg/KJ (40/5 = %prot/%lip), and 23 mg/KJ (44/10 = %prot/%lip) for 3g fingerlings. In the experiment with juveniles, two dietary protein levels (36 and 40%) were combined with two lipid levels (5 and 15%) and two lipid sources (soy bean and capelin). In the experiment with fingerlings, two dietary protein levels (40 and 44%) were combined with three lipid levels (5, 10 and 15%), and the above mentioned two lipid sources. The sources of dietary protein in both experiments were fish meal, meat and poultry meal, soy bean meal, feather meal, fish concentrate protein and soy bean protein. Capelin oil gave the best results as dietary lipid source.

Pereira *et al.* (1987) reported that high protein, high energy diets (51/14 = %prot/%lip) supported better growth performance in 1-6g *S. aurata* when diets with a range of 46 to 51% dietary protein and a fixed 14% lipid content were tested. Fish meal and cod liver oil were the main sources of dietary protein and lipid, respectively.

In a recent work by Takeuchi *et al.* (1991) with red sea bream, the authors found that the optimum combination of dietary protein and lipid for 2-7g fish was 52% and 15%, respectively. White fish meal plus casein and pollack liver oil were the sources of dietary protein and lipid, respectively.

Kissil *et al.* (1981) determined that the minimum dietary vitamin B_6, pyridoxine, requirement of the gilthead sea bream was 1.97 mg/Kg of dry diet.

New (1986) suggests that provided the phosphorus level is kept above about 0.7% of the diet, there is no evidence that any mineral supplementation is necessary in feeds for marine Percoidae which are made from conventional animal feed ingredients. The latter author also suggests provisional dietary vitamin levels in feeds designed for marine Percoidae:

Vitamin	(mg or IU/Kg dry diet)
A	6000 (IU)
B_1(Thiamine)	20
B_2(Riboflavin)	20
B_6(Pyridoxine)	20
B_{12}(Cyanocobolamin)	0.02
Folic acid	5
Inositol	600
PP(Niacin)	150
Pantothenic acid	50
C	200
Choline	2000
D_3(Cholecalciferol)	2500 (IU)
E(α-tocopherol)	200
H(Biotin)	1
K	10

The study of the use of dietary attractants has also been reported for *S. aurata*. Tandler *et al.* (1982) report that *ad-libitum* food intake was significantly increased in this species when basal diet was supplemented with 10 or 20 g/Kg of a synthetic mussel (*Mytilus edulis*) muscle extract.

1.7 The aims of this work

The initial interest in the gilthead sea bream as a potential candidate for intensive aquaculture has lead to a significant research effort devoted to different aspects concerning its nutritional requirements. However, there still exists a lack of detailed research work into major aspects involved.

Quantitative essential amino acid and essential fatty acid requirements, vitamin and mineral requirements, quantitative protein, lipid and carbohydrate requirements as well as the interactions between the different nutrients, digestibility of different feedstuffs and bioenergetics, are among these aspects.

The overall objective of the present work was to carry out orderly nutritional research with *Sparus aurata* on the following:

1) Specific dietary protein requirements.

2) The sparing effect of dietary lipid upon protein.

3) Evaluation of different feedstuffs and the effect of their relative proportion in the diet on nutrient digestibility and energy utilization.

A previously reported work on dietary protein requirements for this species suggests an optimum protein level of 40% for maximum growth in 3g fish (Sabaut and Luquet, 1973). In addition, other authors suggest optimum levels of dietary protein of 40 and 44%, corresponding to dietary lipid levels of 5 and 9%, respectively, for similar size *S. aurata* (45g) (Marais and Kissil, 1979; Kissil and Gropp, 1984)

On the other hand, recent works carried out with gilthead sea bream, and a similar species red sea bream, suggest that high protein, high energy diets could be more suitable for 1-7g fish of these species, with optimum dietary protein levels arround 51-52% and optimum dietary lipid contents of 14-15% (Pereira *et al.*, 1987; Takeuchi *et al.*, 1991).

Thus, there seems to be a certain amount of contradiction between some of these results, particularly when similar nutrient sources were employed in different experimental works.

In addition, the possible protein-sparing effect of dietary lipids in *S. aurata* has not been yet clearly established, and no previous works on digestibility and energy utilization for this species have been reported.

Experiments were conducted with different fish sizes and under particular environmental conditions (Canary Islands).

2. MATERIAL AND METHODS

(GENERAL)

2. MATERIAL AND METHODS

(GENERAL)

2.1 Experimental animals

Four different gilthead sea bream sizes were utilized in six different trials: 0.8g fry, 5g fingerlings, 45g and 60g juveniles, and 90g growers. All were obtained from a local fish farm, DORADA FISH,S.A., located 28 Km. from the marine station where the experiments were carried out.

Healthy fish were selected each time by capturing those fish more actively coming when food was offered. These were then transported in a van equipped with three $1m^3$ tanks half filled with sea water and supplied with bubbled oxygen as required.

2.2 Experimental systems

Three independent experimental tank systems were constructed. All of them consisted of different flow-through fibre-glass tanks. Natural sea water entering

these systems was pumped from a well with two 10 Hp, stainless steel centrifugal pumps to a concrete header tank of 27 m^3 capacity to allow for sedimentation prior to delivery to the tanks.

2.2.1 Tank system I

This system consisted of 24 fibre-glass, 100 litre tanks with P.V.C. pipes. It was maintained with a controlled photoperiod of twelve hours light and twelve hours dark because of poor natural illumination of the unit. The water entered each tank at a rate of 2.5 litre/min. through a tangential pipe to give a circular flow. The outflow was from a circular stand pipe in the bottom of the tank with a framed plastic net cover to avoid fish losses. Effluent water from each tank passed through a drainage channel to the main station outlet. Water passed through a 500 µm cartridge filter prior to entering each tank. The overall system layout and tank shape is shown in Figs. 3a, 3b, and Plate 1.

2.2.2 Tank system II

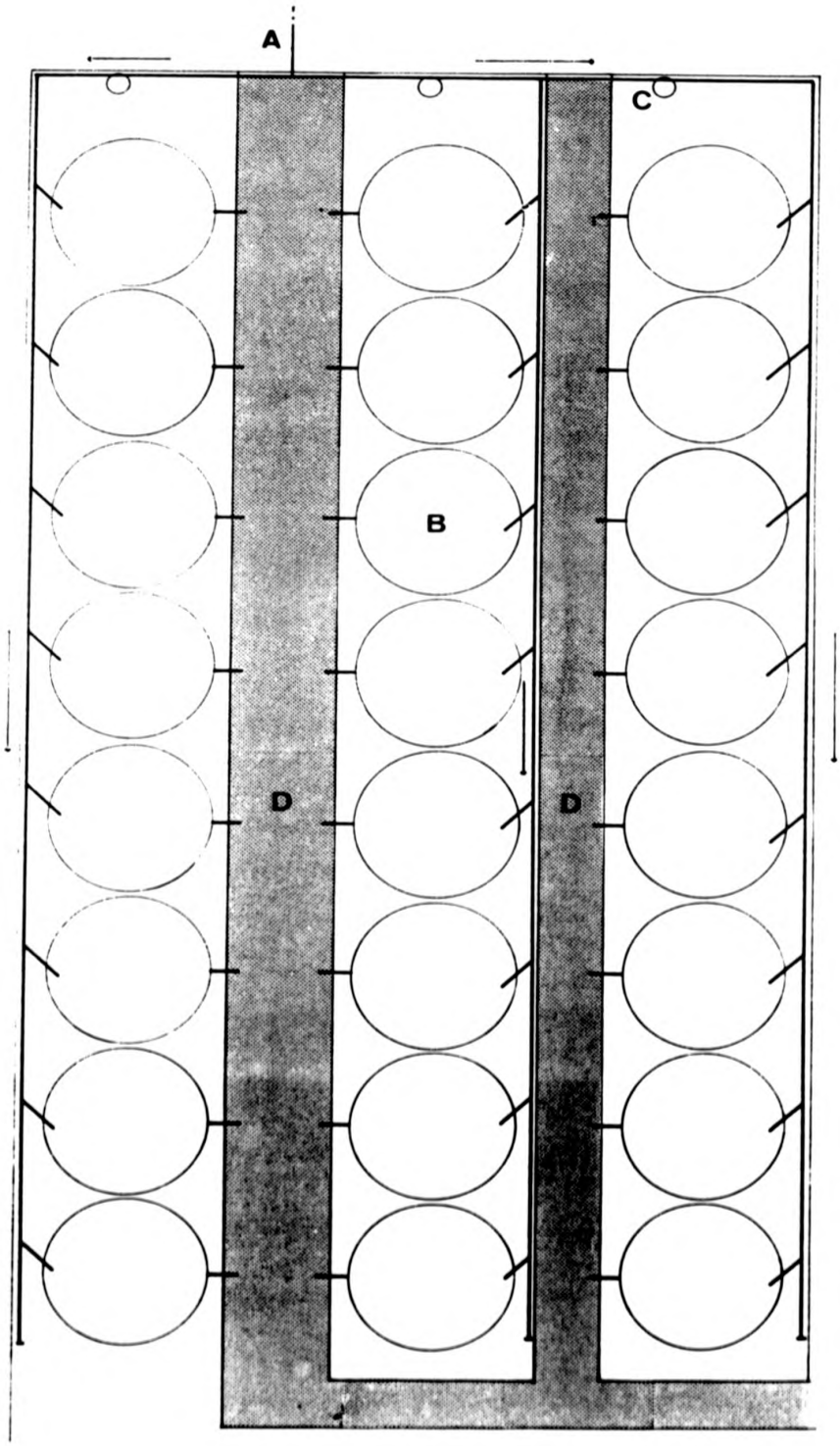
This system was constructed using 15 fibre-glass, 1000 litre tanks with P.V.C. pipes. It was maintained with a natural photoperiod due to good natural illumination of this unit. The water entered each tank at a rate of 10 litre/min. through a tangential pipe. The outflow was from a central stand pipe in the bottom of the tank. Effluent water from each tank was directed through a

Figure 3a. Tank system I:

A) General water inlet. B) Experimental tanks.

C) Cartridge filters. D) Drainage channel.

The arrows show the direction of the water flow.


A
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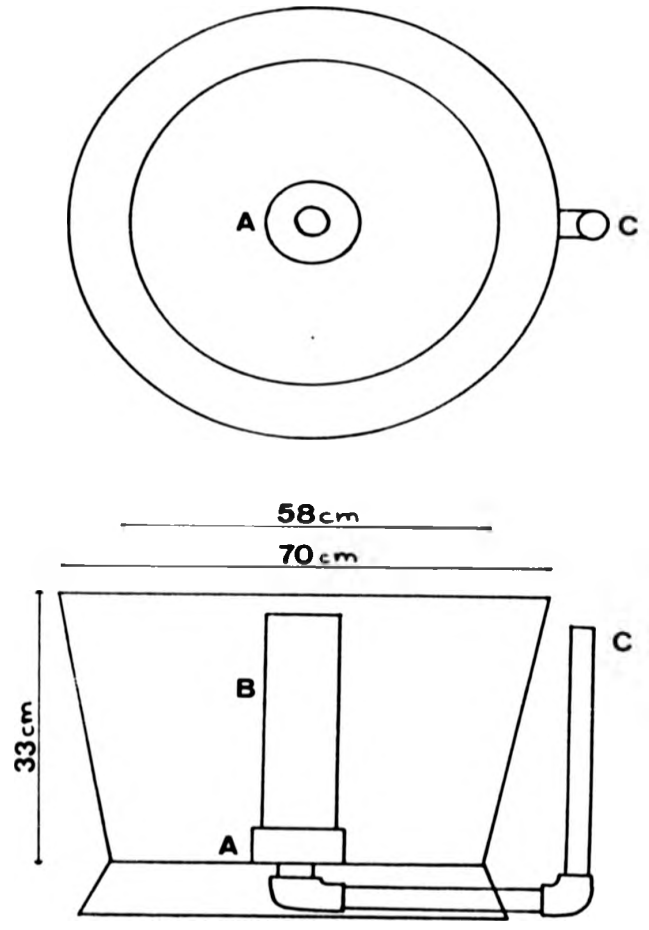


Figure 3b. Tank shape in system I:

A) Circular stand pipe. B) Plastic net

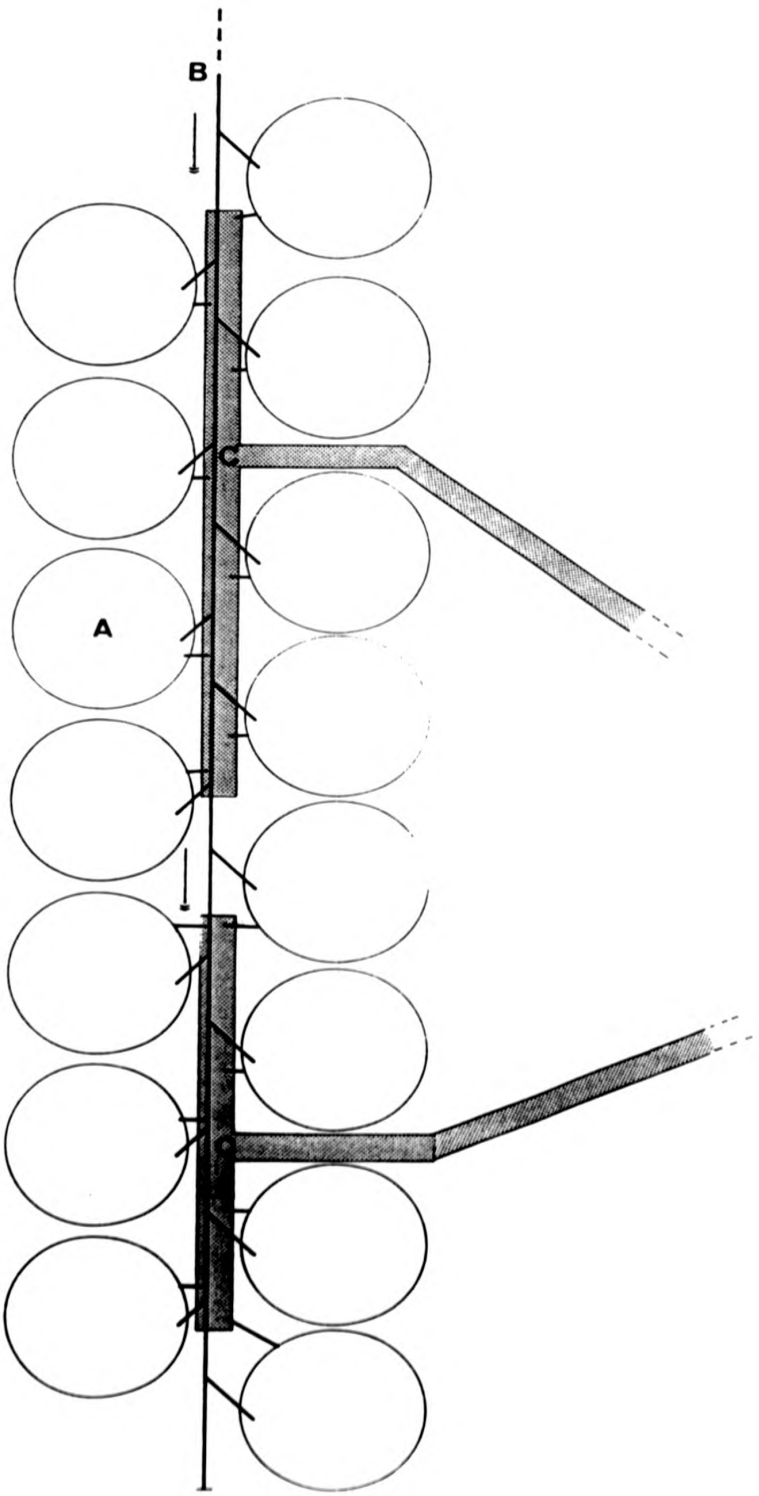
C) Water outlet

drainage channel to the main station outlet. Water entered the system as delivered from the header tank without prefiltering, as it was designed for bigger experimental fish. The overall system layout and tank shape is shown in Figs. 4a, 4b, and Plate 2.

2.2.3 Tank system III

This system consisted of 12 cylindrical, fibre-glass 125 litre tanks with conical bottoms. The water entered each tank at a rate of 6 litre/min. through a tangential pipe. In the deeper zone there was an outlet pipe with a valve, so that all the faeces which were carried by the water flow to this end of the tank were collected. The outflow upwelled through an acrylic settling column on the side of the tank and faeces settled to the base of this column because of their relative density. The faeces could then be collected at the bottom of the column in collection flasks. The faeces-free effluent water from each tank was drained from the top of the sedimentation columns and directed through a drain pipe and drain channels to the main station outlet. Water entered the system as delivered from the header tank. The overall system and tank shape is shown in Figs. 5a, 5b, and Plate 3.

Figure 4a. Tank system II:
A) Experimental tanks. B) General water inlet. C) Drainage channel.
The arrows show the direction of the water flow.


B
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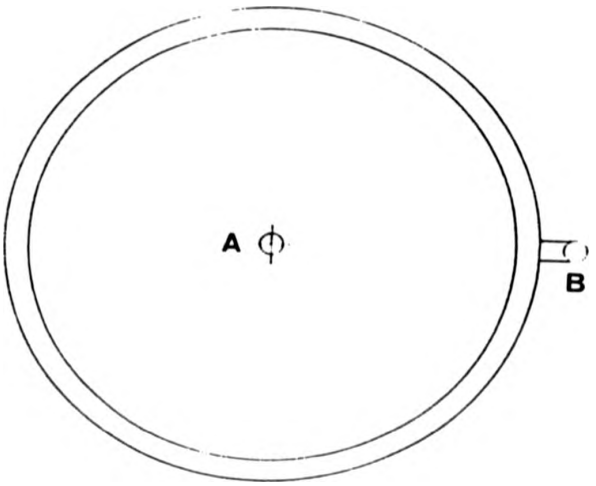
A



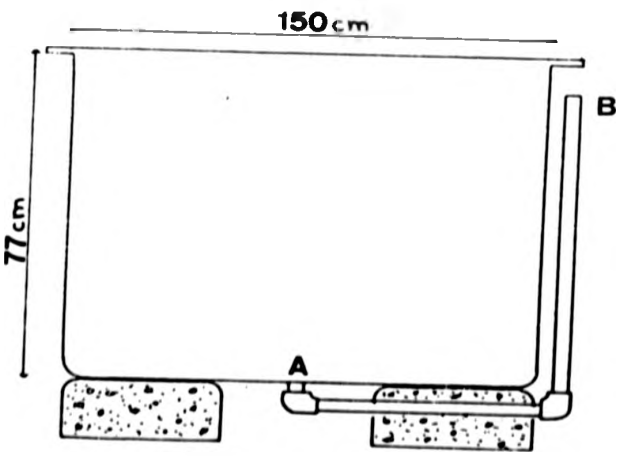
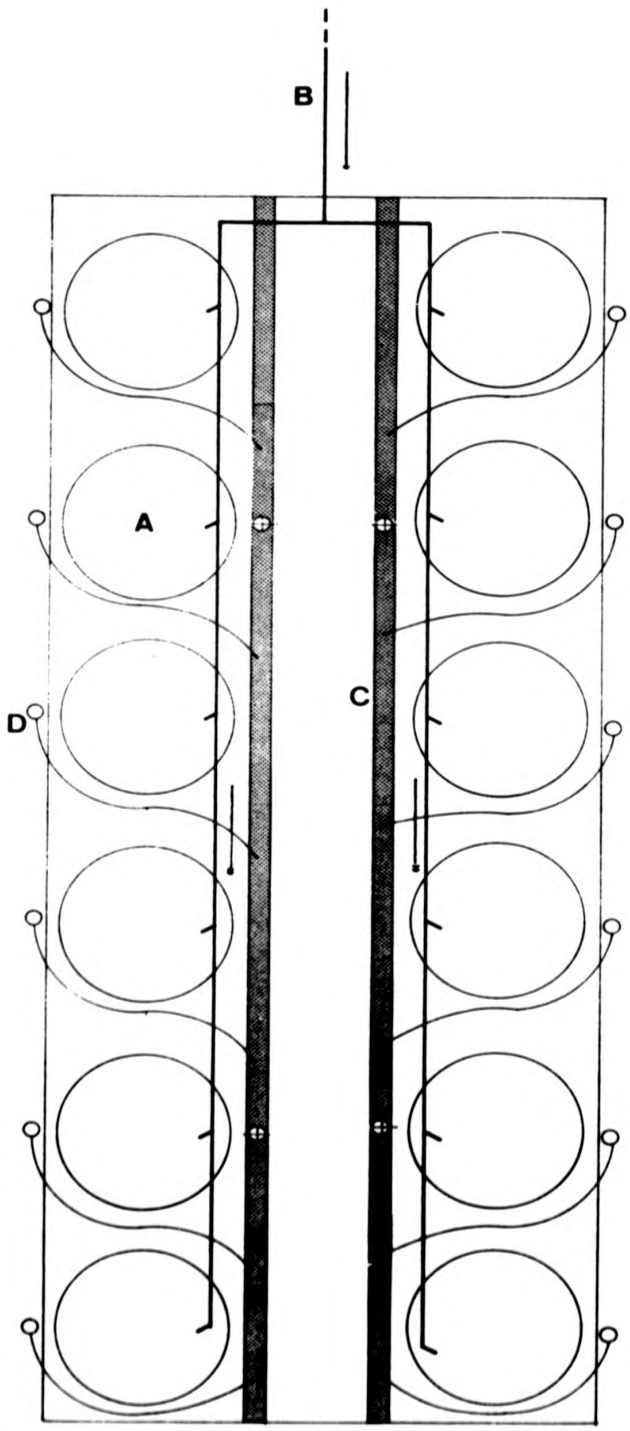


Figure 4b. Tank shape in system II:

A) Circular stand pipe. B) Water outlet

Figure 5a. Tank system III:
A) Experimental tanks. B) General water inlet. C) Drainage channel. D) Settling column.
The arrows show the direction of the water flow.


B
A
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D

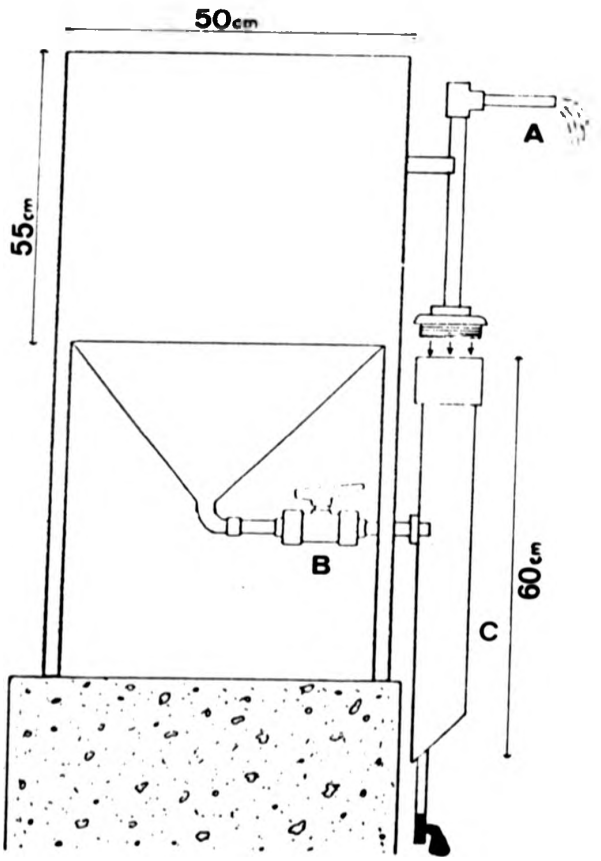


Figure 5b. Faeces collection tank:
A) Water outlet. B) Valve. C) Settling column.

Plate 1. Experimental tank system I.

Plate 2. Experimental tank system II.

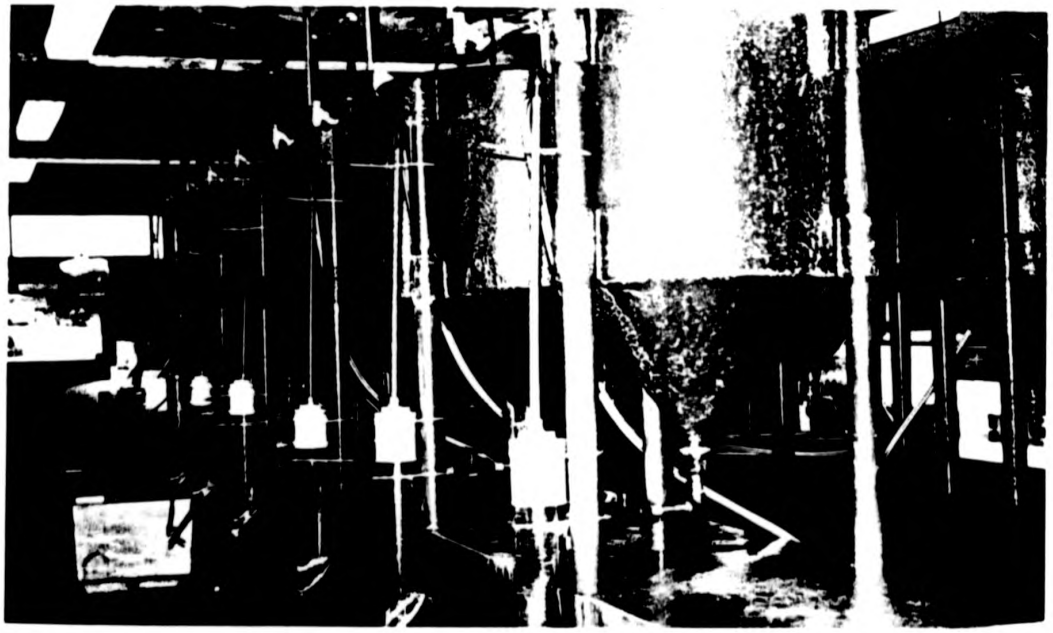


Plate 3. Experimental tank system III.

2.3 Environmental parameters

The water temperature was recorded twice a day with a Crison digital portable thermometer (Mod. 637). Dissolved oxygen levels and pH values were measured every three days with a Ciba-Corning portable PH, conductivity and dissolved oxygen meter (Mod. M-90). Salinity was measured twice a week with an Atago portable refractometer. Nitrite values were estimated every three days using the method described by Parsons *et al.* (1985). This parameter was measured as an alternative to ammonia because the appropriate technique was not available by the time the experiments were carried out, and high ammonia levels were unlikely to occur as flow-through systems were always used.

2.4 Experimental diets

Based on reported work by Luquet and Sabaut (1973), where the authors studied some essential amino acid requirements for gilthead sea bream, and on direct correlation found between the pattern of amino acids in pig and chicken tissues as a percentage of the total essential amino acids and the dietary requirements for amino acids (Boorman, 1980) and described for some well studied fish species (Cowey and Tacon, 1981; Tacon, 1990), the essential amino acid profile of *S. aurata* fingerlings was used as a reference for dietary formulation.

The amino acid profile of *S. aurata* was obtained by killing a number of reared fingerlings of average weight 1.05g by overdose of benzocaine (1:400). These were then dried in an oven at 105°C overnight, the fat was extracted by soxhlet with petroleum ether and the protein was then hydrolysed with 6M hydrochloric acid. Amino acids were then analysed in a Chromakon amino acid analyzer (Mod. 500) as per manufacturers specifications.

Table 5a shows the carcass amino acid profile as a percentage of the total essential amino acids for sea bream. This was used for establishing the relationships between carcass composition and requirements. Table 5b also shows the amino acid profile of *Sardina pilchardus* meal, which was used as the protein source during the trials, as represents the amount of amino acids supplied in diets containing 40% and 55% protein, for reference. (Background analysis and data calculation are summarized in Appendix I).

When comparing these data with those reported by Luquet and Sabaut (1974), the quantitative requirements of three determined essential amino acids for sea bream appear to be covered by the experimental diets used in the present work. Thus, when expressed as a percentage of dietary protein, the amount of arginine offered was 5.9 (< 2.6); lysine, 9.0 (5.0) and methionine plus cystine, 4.17 (4.0); values in parenthesis representing those reported by Luquet and Sabaut (1974) for the gilthead sea bream.

Table 5a
Relationship between carcass essential amino acid (EAA) profile of *S. aurata* and its EAA requirement.

Percentage of EAA as % of total EAA in sea bream fingerlings carcass.		Calculated EAA requirements of sea bream fingerlings for diets containing A (40%) and B (55%) protein, as % of dry diet.	
		A	B
Arginine	12.71	1.74	2.39
Histidine	5.23	0.73	1.00
Isoleucine	10.74	1.50	2.07
Leucine	15.01	2.10	2.89
Lysine	15.70	2.20	3.02
Methionine	5.71	0.80	1.10
Phenylalanine	7.21	1.01	1.39
Threonine	10.52	1.47	2.03
Valine	9.35	1.31	1.80
Cystine(*)	2.00	0.28	0.38
Tyrosine(*)	6.11	0.85	1.18

(*) Non-essential amino acids
Basis of analysis: 1.05 $\pm$ 2.9 g average body wt. fingerlings.

Table 5b
The EAA profile of the sardine meal (*S. pilchardus*) as percentage of total EAA, utilized as sole food (A), and amount of EAA supplied in diets containing B (40%), and C (55%) protein as percentage of dry diet.

	A	B	C
Arginine	10.17	2.35	3.23
Histidine	5.92	1.36	1.88
Isoleucine	11.70	2.70	3.71
Leucine	14.96	3.45	4.75
Lysine	15.58	3.60	4.94
Methionine	5.23	1.21	1.66
Phenylalanine	8.11	1.87	2.57
Threonine	10.39	2.40	3.30
Valine	9.63	2.22	3.06
Cystine(*)	1.99	0.46	0.63
Tyrosine(*)	6.32	1.46	2.01

(*) Non-essential amino acids.
NB. Tryptophan, as essential amino acid, was not recovered by the analytical technique used.

The experimental diets were formulated using sardine meal as the only source of protein. Table 6 shows the fatty acid profile of the sardine oil which was used as the main source of lipids in the experimental diets. Oil samples were transmethylated overnight using nonadecanoid acid (19:0) as internal standard (Christie, 1982). Methyl esters were extracted with hexane:diethyl ether (1:1, v/v) and purified by thin-layer chromatography. Analyses of fatty acid methyl esters were performed on a capillary gas chromatograph using on-column injection and thermal gradient.

The diet ingredients were mixed with a Danamix mixer (Mod. BM 330) for at least 20 minutes and later the oil was incorporated with a small amount of distilled water to obtain a crumble mixture. The mixture was then pelleted by extrusion in a 2 HP Mobba pellet mill (Mod. 8.3) prepared to produce pellets with the required diameter for each experiment.

The pellets were then dried at 40°C in a forced air convection dryer and the final diet was stored in air-tight containers in a freezer at -20°C. Small portions of each diet were weekly placed in the fridge (4°C) and weighed out daily as required for feeding.

Dried samples of the prepared diets were taken for triplicate proximate analysis. Values for gross energy (GE) and metabolizable energy (ME) in diets were obtained by calculation (with the exception of diets used in digestibility

Table 6
Fatty acid composition (wt %) of sardine oil utilized in diets as main source of lipid

14:0	8.4
15:0	0.6
16:0	19.5
16:1(n-7)	12.1
16:2	1.7
16:3	2.1
16:4	3.2
17:0	0.5
18:0	3.2
18:1(n-9)	7.3
18:1(n-7)	3.7
18:2(n-6)	1.0
18:3(n-6)	0.6
18:3(n-3)	0.6
18:4(n-3)	3.5
20:0	0.6
20:1(n-9)	1.3
20:2	0.3
20:3	0.2
20:4(n-6)	0.7
20:4(n-3)	0.9
20:5(n-3)	16.3
22:1(n-11)	0.6
21:5	0.7
22:5(n-3)	1.3
22:6(n-3)	4.3
Unknowns	4.8

Total PUFA (n-3,6 and 18, 20, 22C): 29.2 (wt%)
Total HUFA (n-3 and 20,22C): 22.8 (wt%) Essential to marine fish (Watanabe,1982)

Total saturates ..... 32.8 (wt%)
Total monounsaturates .. 25.0 "
Total polyunsaturates .. 37.4 "

trials), based on the assumption that protein had an energy value of 23.4 KJ/g (18.85 KJ/g for ME) (Smith, 1971), carbohydrates had an energy value of 17.2 KJ/g (14.62 KJ/g for ME) (Chiou and Ogino, 1975) and dietary lipids had an energy value of 39.8 KJ/g (35.66 KJ/g for ME) (Austreng, 1978; Cho *et al.*,1982; Brafield, 1985).

Tables 7 and 8 show the mineral and vitamin mixtures used in the experiments, which were prepared with carboxy methyl cellulose (sodium salt) as carrier. Commercial mixtures were employed after experiment III for practical reasons, as considerable ammounts of experimental diets were produced for experiments IV, V and VI.

2.5 Chemical analysis

Fish carcasses, diets and faeces were analyzed for crude protein using the Micro-Kjeldhal technique with a Tecator/Kjeltec System 1003 distilling unit (AOAC, 1985). The fat content was determined by extracting dried samples using a soxhlet apparatus and petroleum ether (40-60°). Crude fibre content was determined by the digestion method with diluted H_2SO_4(0.225 N) and NaOH (0.313 N) (AOAC, 1985). Ash content was determined by heating a pre-weighed sample within a silica crucible in a muffle furnace at 450°C for 12 hours. Moisture was determined by drying a weighed sample in a drying oven at 105°C for 24 hours (AOAC, 1985).

Table 7
Mineral and vitamin mixtures used in all diets (Exp.I,II,III). (*)

Vitamin Mixture(1) (mg/Kg or IU/Kg of dry diet)		Mineral Mixture(2) (g/Kg of dry food)	
Thiamine(B_1)	40.0	$(H_2PO_4)Ca$	1.6050
Riboflavin(B_2)	50.0	$Ca\ CO_3$	4.0000
Pyridoxine(B_6)	40.0	$FeSO_4\ .7H_2O$	1.5000
Calcium Pantothenate	117.0	$MgSO_4\ .7H_2O$	1.6050
Nicotinic Acid	200.0	K_2HPO_4	2.8000
Biotin (H)	1.0	$Na_2PO_4\ .H_2O$	1.0000
Folic Acid	10.0	$Al_2(SO_4)_3.6H_2O$	0.0200
Cyanocobalamin(B_{12})	0.5	$ZnSO_4\ .7H_2O$	0.2400
Choline Chloride	2700.0	$CuSO_4\ .5H_2O$	0.1200
Myo-Inositol	600.0	$MnSO_4\ .H_2O$	0.0800
Ascorbic Acid(Vit C)	1000.0	KI	0.0200
Alpha Tocopherol(Vit E)	250.0	$CoSO_4\ .7H_2O$	0.0800
Menadione (K_3)	20.0		
Cholecalciferol(D_3) IU	2000.0		
Ethoxyquin	100.0		
Retinol acetate (A) IU	5000.0		

(*) New, 1986; Higuera, personal communication.

Table 8
Mineral and vitamin mixtures used in all diets (Exp.IV,V,VI). (*)

Vitamin Mixture (mg/Kg or IU/Kg of dry diet)		Mineral Mixture (mg mineral/Kg of dry diet)	
Retinol (Vit A)	IU 50000.0	Mn	9.3
Cholecalciferol (D_3)	IU 2000.0	Cu	0.9
Alpha Tocopherol (Vit E.	IU 300.0	Co	2.2
Thiamine (B_1)	37.0	I	2.0
Riboflavin (B_2)	48.0	Zn	44.0
Pyridoxine (B_6)	20.0	Ethoxyquin	130.0
Cyanocobalamin (Vit B_{12})	0.1		
Folic Acid	10.0		
Calcium Pantothenate	74.0		
Menadione (K_3)	11.0		
Ascorbic Acid (Vit C)	240.0		
Myo-Inositol	337.0		
Biotin (H)	0.5		
Choline Chloride	1700.0		
Nicotinic Acid	300.0		

(*) Commercial mixtures supplied by A.T.P., A/S

Chromic oxide content was determined using the wet acid method proposed by Furukawa and Tsukahara (1966). Gross energy content of fish carcasses, diets and faeces was determined using a PARR oxygen bomb calorimeter (Mod. 1261).

2.6 Nutritional formulae

The following formulae were utilized in the nutritional assessment of the effects of dietary protein level and protein to energy ratio on growth and performance of *S.aurata*. Formulae employed in assessment of digestibility and nutritional energetics are also shown.

GROWTH

INDIVIDUAL WEIGHT GAIN (IWG g(mg)/day)

$$IWG = \frac{(W/n)}{T}$$

Where W = total population weight gain over time T (days) and n individuals were present at time T.

WEIGHT GAIN (%)

$$\text{Weight gain (\%)} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

SPECIFIC GROWTH RATE (SGR %/day)

SGR measures the average change of fish weight in percent per day.

$$SGR = \frac{(\text{Log}_e \text{ Final weight} - \text{Log}_e \text{ Initial weight})}{\text{Time (days)}} \times 100$$

FEED EFFICIENCY

INDIVIDUAL FOOD FED (IFF g(mg)/day)

$$IFF = \frac{(I/n)}{T}$$

Where I = total food fed over time T (days) and n individuals were present at time T.

FOOD CONVERSION EFFICIENCY (FCE)

$$FCE = \frac{\text{Individual weight gain (g, mg)}}{\text{Individual food intake (g, mg)}}$$

PROTEIN EFFICIENCY

PROTEIN EFFICIENCY RATIO (PER)

PER measures the ability of fish to utilize dietary protein (Osborne et al., 1919)

$$PER = \frac{\text{Individual weight gain (mg)}}{\text{Individual protein fed in mg}}$$

NITROGEN AND LIPID UTILIZATION

APPARENT NET NITROGEN UTILIZATION (%)

App.N.U. measures the capacity of the fish to utilize nitrogen in the diet.

$$\text{App.N.U.} = \frac{\text{Nitrogen deposition (mg)}}{\text{Nitrogen intake (mg)}} \times 100$$

$$\text{App.Lipid Utilization} = \frac{\text{Lipid deposition (g)}}{\text{Lipid intake (g)}} \times 100$$

DIGESTIBILITY

$$\text{TOTAL DIGESTIBILITY(\%)} = 100 - \left(100 \times \frac{\text{\% indicator in food)}}{\text{\% indicator in faeces}}\right.$$

APPARENT NUTRIENT DIGESTIBILITY (AND %)

$$\text{AND(\%)} = 100 - \left(100 \times \frac{\text{\% indicator in food}}{\text{\% indicator in faeces}} \times \frac{\text{\% nutrient in faeces)}}{\text{\% nutrient in food}}\right.$$

NUTRITIONAL ENERGETICS

Digestible energy (DE) = Gross energy in food - Gross energy in faeces.

Recovered energy (RE) = Final carcass gross energy - Initial carcass gross energy.

Energy retention efficiency (ERE) = RE / DE

Estimation of Non-faecal losses:

Losses of digested, absorbed nitrogen occur mainly through the gills and in the urine with negligible amounts from surface losses as mucous and scales (Cho, 1987). Therefore, total non-faecal nitrogen loss, branchial and urinary, is estimated by the difference between digested nitrogen and recovered nitrogen as shown in the following expressions:

$$ZN + UN = DN - RN$$

$$ZE + UE = (ZN + UN) \times 24.9 \text{ Kj/g N}$$

$$ZN + UN = 24.9 \times (\text{Digestible N intake} - \text{Recovered N})$$

Where
ZN = branchial N loss
UN = urinary N loss
DN = digestible N intake
RN = recovered tissue N
ZE = branchial energy loss
UE = urinary energy loss
ME = metabolizable energy
DE = digestible energy

Note. 24.9 KJ/g N was used to estimate the energy loss of the non-faecal nitrogen excretion based on the assumption that the major part of the nitrogen (>85%) is excreted as ammonia (Cho, 1987).

2.7 Statistical analysis

One-way analysis of variance, Tukey's range test, Kruskal-Wallis test and Student's test were employed in evaluating the significance of the experimental results (Zar, 1984).

A dose-response analysis was also employed to determine the nutritional requirements for protein (Zeitoun *et al.*, 1976).

3. DIETARY PROTEIN REQUIREMENTS OF *Sparus aurata* AT TWO DIFFERENT SIZES

3.1 INTRODUCTION

Proteins represent the largest chemical group in the animal body with the exception of water. The whole fish carcass contains on average 75% water, 16% protein, 6% lipid and 3% ash (Tacon, 1990).

Protein synthesis requires dietary amino acid supply. At maintenance level the fish requires protein for replacement of tissues such as intestinal epithelial cells, enzymes and hormones, which are vital for the proper function of the body and are recycled quite rapidly. As the tissue protein content is high it is obvious that protein requirements will increase with increased rates of tissue synthesis (e.g. during growth or gametogenesis) (Fauconneau, 1985).

If adequate protein is not provided in the diet, there is a rapid reduction or cessation of growth or a loss of weight because the animal withdraws protein from some tissues to maintain the functions of more vital ones. On the other hand, if too much protein is supplied, proportionally less will be used to make new protein and the rest will be metabolized to produce energy (N.R.C., 1983).

Thus, the protein requirement of any animal species is one of the major questions to resolve before being able to prepare balanced diets.

The study of dietary protein requirements in fishes has been almost entirely based on studies comparable to those conducted with terrestrial farm

animals. This involves laboratory based feeding trials where the animals are kept in a controled environment at high density and having no access to natural food organisms (Tacon, 1990).

Dietary protein requirements of fish are normally investigated by feeding the animals different balanced diets containing graded levels of a high quality protein (Tacon, 1990), and from the resulting dose-growth response curve the protein requirement is usually obtained by an "Almquist plot" (Zeitoun *et al.*, 1976). Other criteria such as indexes of protein utilization in response to the above treatments (Ogino, 1980), or the use of different ratios of protein to dietary energy to investigate dietary protein requirements (Tacon, 1990), are different approaches used by some workers today.

Many of the results on dietary protein requirements for various fish species shown in Table 2 (Section 1.2.2) were obtained using semi-purified or purified test diets (De Long *et al.*, 1958; Nose and Arai, 1972; Yone *et al.*, 1974; Garling and Wilson, 1976; Dabrowski, 1977; Sen *et al.*, 1978; Takeuchi *et al.*, 1979; Kanazawa *et al.*, 1980; Winfree and Stickney, 1981; Shi *et al.*, 1988). However, it is broadly recognized that purified proteins, such as casein, are deficient in certain essential amino acids (Jauncey, 1982). Furthermore, when casein-based diets supplemented with crystaline methionine (of which casein is deficient) were fed to rainbow trout, the absorption of this amino acid took place sooner than the rest of the dietary amino acids, diminishing growth and food conversion efficiency (Higuera, personal communication). This is because

optimal protein synthesis requires that all amino acids (whether they are derived from whole proteins or amino acid supplements) are presented simultaneously to the tissue. If such an equilibrium is not achieved, amino acid catabolism takes place with consequent loss of growth and feed efficiency. Other fish species have also shown a more rapid absorption of free amino acids than that of amino acids from whole proteins (Plakas *et al.*, 1980; Yamada *et al.*, 1982).

Up to date, the only work on the quantitative protein requirement of the gilthead sea bream (Sabaut and Luquet, 1973) was performed using semi-synthetic diets. Casein supplemented with a mixture of synthetic amino acids, in order to obtain a balance similar to hen's egg protein, was used as the dietary protein source.

The above authors reported a minimum dietary protein level for optimum growth of 40% for 3g initial body weight fish. Dietary lipid levels were maintained at 8%, consisting of a mixture of soy bean oil (5.7%) and cod liver oil (2.3%).

In a work reported by Kissil and Gropp (1984), optimum dietary protein and lipid for 3g *S.aurata* fingerlings were found to be 44% and 10% respectively when a mixture of fish meal, meat and poultry meal, soy bean meal, feather meal, fish protein concentrate and soy bean protein was used as the protein source, and capelin fish oil as the lipid source. With these ingredients, 40% protein and 5% lipid were found optimum for 45g juveniles.

On the contrary, other works carried out with 1-7g gilthead sea bream and red sea bream using fish meals and fish oils in the diets reported optimum dietary protein levels around 51-52% for both species (Pereira *et al.*, 1987; Takeuchi *et al.*, 1991).

The aim of the present study was to determine the quantitative dietary protein requirement of *S.aurata* by varying the levels of protein in diets, using sardine meal and sardine oil as protein and lipid sources, respectively. The study was designed to provide a basis to discuss some of the contradictory results reported for this species, particularly when similar nutrient sources were employed in different experimental works, and also to provide preliminary data for the next chapter, where dietary protein requirements were studied in relation to optimum dietary protein to energy ratios.

3.2 MATERIALS AND METHODS

For the two different trials (I and II), sea bream fry and juveniles of mean body weights 0.8g and 59.6g were employed, respectively. Experimental fish were stocked at 30 fry per tank and 15 juveniles per tank, three tanks per treatment.

Prior to starting the experiments, both fry and juveniles were acclimated for one week, feeding them with diets containing 39.3% and 41.0% protein at a

rate of 3% and 2% of body weight/day, respectively. Feeding rates in fry were significantly lower during the acclimation period as fish demand was lower than that observed after the first week, when the above rate (3%) was increased to 6% of body weight/day. At the start of the experiments 100 fry and 10 juveniles were killed by an overdose of benzocaine and stored at -20°C for subsequent chemical analysis.

Tank system I (Section 2.2.1) was utilized in both trials and Table 9 shows the mean values obtained for the environmental parameters recorded during the present experiments (As detailed in Section 2.3). All values were within the optimum ranges for this species (Section 1.6).

Variations in the dietary protein level were achieved by replacement of fish meal with starch and dextrin in order to produce approximately isoenergetic diets. The objective in the first experiment was to obtain diets containing a range of dietary protein from 35 to 65% and a fixed lipid content of 9%, and a range of 30 to 60% protein and a fixed 9% lipid in the second trial. Proximate analysis (Section 2.5) of the sardine meal, including determination of moisture, crude protein, ether extract, ash and nitrogen free extractive (NFE) was performed prior to diet formulation. Table 10 shows the composition of the experimental diets for the experiment with fry, and Table 11 shows the composition of the diets for the experiment with juveniles.

Table 9
Environmental parameters recorded during protein level experiments with fry and juveniles.

FRY	
Temperature	22.18°C ± 0.82
pH	8.11 ± 0.02
Dissolved oxygen	6.7 ± 0.55 mg/l
Salinity	36.6 ± 0.07 ppt
Nitrite	0.013 ± 0.009 mg/l
JUVENILES	
Temperature	21.19°C ± 1.18
pH	8.08 ± 0.06
Dissolved oxygen	6.3 ± 0.62 mg/l
Salinity	36.6 ± 0.05 ppt
Nitrite	0.028 ± 0.009 mg/l

Table 10
Composition of the experimental diets used in the experiment with sea bream fry.
Proximate analysis on a moisture free basis, with the calculated energy and protein:energy ratio.

INGREDIENTS (%)	DIET NUMBER						
	1	2	3	4	5	6	7
Sardine meal(1)	46.37	54.10	61.83	69.56	77.29	85.02	92.75
Corn dextrin	9.30	7.87	6.45	5.00	3.60	2.15	0.75
Corn starch	27.90	23.62	19.35	15.00	10.80	6.45	2.25
Sardine oil	4.48	3.73	2.97	2.22	1.47	0.71	0.00
C.M.Cellulose(2)	7.95	6.68	5.40	4.32	2.84	1.67	0.25
Vitamin Premix(3)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral Premix(3)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
NUTRIENT CONTENT (%)							
Crude protein	33.77	39.28	45.06	50.14	55.56	63.00	67.29
Ether extract	9.32	9.20	9.18	8.77	8.94	8.61	9.16
Ash	11.09	12.13	13.11	13.37	14.37	15.49	16.33
Fibre	0.14	0.22	0.12	0.26	0.70	0.13	0.13
NFE (4)	45.89	39.39	32.65	27.72	21.13	12.90	7.22
GE (MJ/Kg)(5)	19.53	19.68	19.88	20.06	20.28	20.49	20.74
ME (MJ/Kg)(6)	16.38	16.44	16.54	16.63	16.75	16.83	17.00
P:E ratio (7)	17.29	19.96	22.66	24.99	27.40	30.75	32.44

(1) Proximate analysis (% dry wt.): Crude protein: 72.7, Ether extract: 8.5, Fibre: 9.7, Ash: 17.2.
(2) Carboxy methyl cellulose (Sodium salt)
(3) Table 7, Section 2.4
(4) NFE = Nitrogen free extractives, calculated as 100 - (% prot. + % lip. + % ash)
(5) GE = Gross energy content.
(6) ME = Metabolizable energy content.
(7) P:E = Protein to energy ratio in g protein/MJ of GE.

Table 11
Composition of the experimental diets used in the experiment with sea bream juveniles.
Proximate analysis on a moisture free basis, with the calculated energy and protein:energy ratio.

INGREDIENTS (%)	DIET NUMBER						
	1	2	3	4	5	6	7
Sardine meal(1)	37.48	44.98	52.47	59.97	67.47	74.96	82.46
Corn dextrin	12.36	10.63	8.91	7.18	5.45	3.72	1.99
Corn starch	37.09	31.90	26.72	21.53	16.35	11.17	5.99
Sardine oil	6.07	5.49	4.90	4.32	3.73	3.15	2.56
C.M.Cellulose(2)	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin premix(3)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix(3)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Indicator (Cr_2O_3)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
NUTRIENT CONTENT (%)							
Crude protein	32.41	36.81	41.03	45.27	50.89	56.14	62.20
Ether extract	9.57	9.41	9.03	8.54	8.61	8.64	9.47
Ash	9.43	10.49	11.56	12.69	13.84	15.15	16.46
Fibre	0.60	0.53	0.60	0.41	1.09	0.51	0.35
NFE (4)	48.59	43.29	38.38	33.50	26.66	20.07	11.87
GE (MJ/Kg) (5)	19.79	19.85	19.85	19.82	19.99	20.11	20.46
ME (Mj/Kg) (6)	16.62	16.62	16.56	16.48	16.56	16.60	16.84
P:E ratio (7)	16.38	18.54	20.67	22.84	25.46	27.92	30.40

(1) Proximate analysis (% dry wt.): Crude protein: 66.7, Ether extract: 7.81, Fibre: 9.5, Ash: 16.7.
(2) Carboxy methyl cellulose (Sodium salt).
(3) Table 7, Section 2.4.
(4) NFE = Nitrogen free extractives, calculated as 100 - (% prot. + % lip. + % ash)
(5) GE = Gross energy content.
(6) ME = Metabolizable energy content.
(7) P:E = Protein to energy ratio in g protein/Mj of GE.

The diameter of pellets was 2mm for the juveniles, with a length: diameter ratio of 2:1, and the latter broken into smaller pieces according to the size of the fish, in the case of fry (Average size: 1mm).

The feeding regimes used throughout both trials were 6% and 2% body weight per day, respectively, the frequency of feeding was maintained at 5 times a day, 6 days a week, and twice on sundays to optimize food conversion efficiency. The food was given taking care to provide a small amount of food at a time, to be sure that the fish ate all the diet offered. The trials were continued for 54 days with gilthead sea bream fry and for 82 days with juveniles.

At the start and at the end of the experiments, fish were individually weighed under anaesthesia (Methylpropanol -3- Chlorine, 7ml/10l) to the nearest two decimal places (0.02g) on a Mettler top-pan balance (Model PE-3600). At subsequent fortnightly intervals, fish were batch weighed in a bucket containing pre-weighed water to avoid stressfull handling and anaesthesia. At the end of the experiments, fish were dried in order to obtain total moisture and later the carcasses were finely ground for chemical analysis.

Fish mortality was recorded daily and the weights of dead fish were subtracted from the total in order to adjust the daily amount of food offered. As dead fish were removed every early morning, they were usually intact. All fish dead before every weighing were excluded from records, and this was applied in all experiments.

3.3 RESULTS

3.3.1 EXPERIMENT I (Fry)

3.3.1.1 Growth

The growth response of *S.aurata* fry over the experimental period to different dietary protein levels is shown in Figure 6. The best growth response, in terms of final body weight, was observed for fish consuming diet 5 (55.6% protein), with significant ($p < 0.05$) differences between this protein level and the other diets following in the order: 6 (63.0% protein) > 4 (50.1% protein) > 7 (67.3% protein). No significant differences ($p < 0.05$)were found in final body weight between the fish fed the lower protein diets 1, 2 and 3 (33.8%, 39.3% and 45.1% protein, respectively). Growth supported by these diets was significantly ($p < 0.05$) lower than supported by the higher protein diets (Table 12).

A similar response was also observed on the basis of specific growth rate and percentage weight gain, the maximum value always being found for diet 5 (Table 12).

A trend of increment in specific growth rate with dietary protein level was observed (Figure 7). This trend was not maintained above 55.6% protein, where the specific growth rate declined.

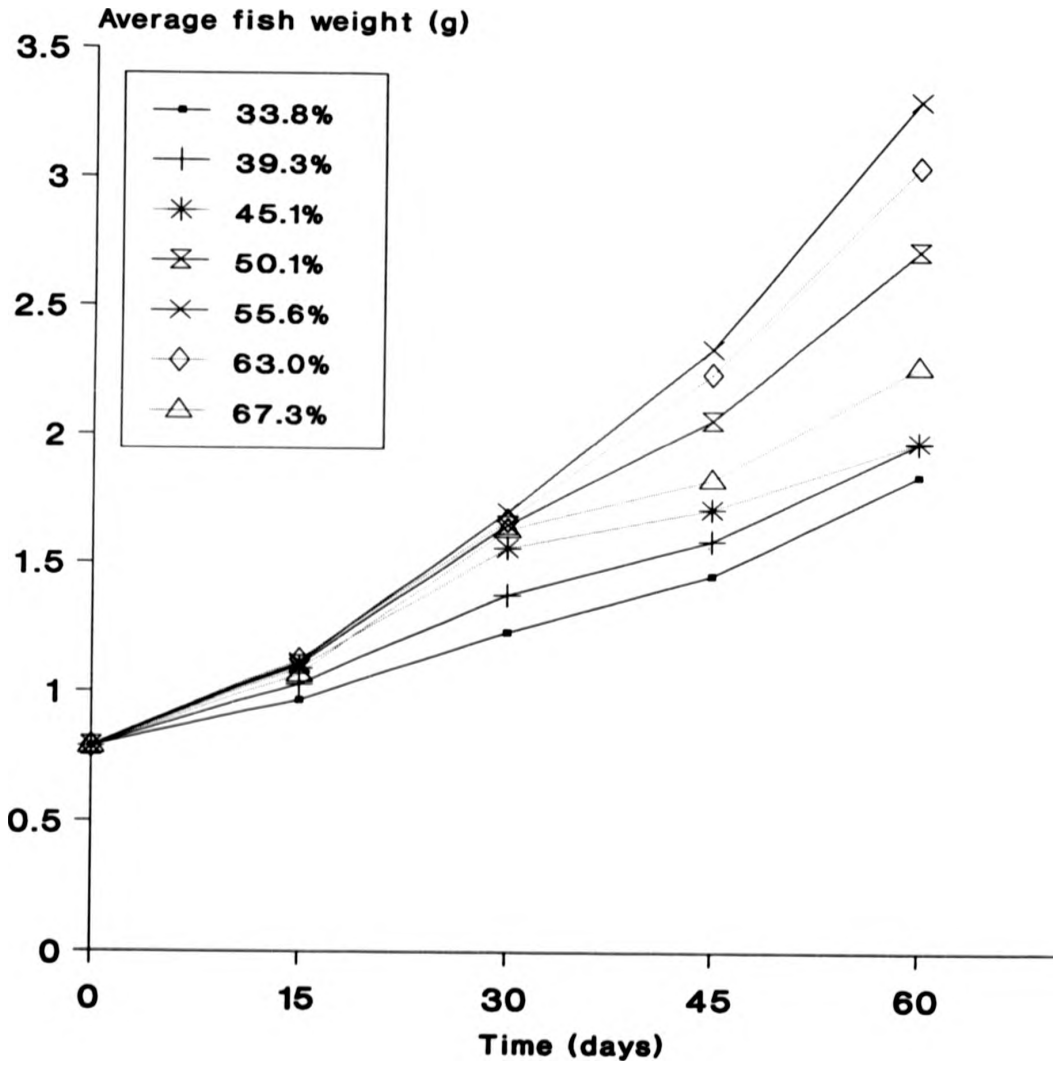


Figure 6. Growth response of S.aurata fry over the experimental period at different dietary protein levels.

Table 12
Mean growth performance, feed utilization efficiency and carcass composition of S.aurata fry fed the experimental diets.

MEAN VALUE RESULTS (Std. error in parenthesis)	DIET NUMBER						
	1	2	3	4	5	6	7
PROTEIN IN FOOD (%)	33.8	39.3	45.1	50.1	55.6	63.0	67.3
INITIAL BODY WT.(g) (0.09)	0.79	0.79	0.79	0.79	0.79	0.79	0.79
FINAL BODY WT. (g)*	1.85^{a} (0.03)	1.98^{a} (0.04)	1.98^{a} (0.04)	2.71^{b} (0.06)	3.31^{c} (0.04)	3.06^{d} (0.06)	2.27^{e} (0.05)
WEIGHT GAIN (%)*	134.57^{a} (3.59)	149.74^{a} (5.55)	151.64^{a} (5.29)	244.78^{b} (7.10)	320.42^{c} (5.16)	287.12^{d} (8.08)	189.08^{e} (6.16)
SPECIFIC GROWTH RATE (%/day)*	1.41^{a} (0.03)	1.50^{a} (0.04)	1.53^{a} (0.04)	2.05^{b} (0.04)	2.40^{c} (0.02)	2.23^{d} (0.03)	1.74^{e} (0.04)
FOOD FED (mg/day)	45.87^{a} (6.16)	45.34^{a} (6.56)	41.66^{a} (9.72)	54.22^{a} (9.75)	63.55^{a} (9.72)	59.76^{a} (9.72)	56.28^{a} (10.29)
WEIGHT GAIN (mg/day)	18.61^{a} (1.27)	20.59^{a} (2.47)	25.48^{ab} (3.40)	35.09^{ab} (4.65)	40.58^{b} (5.83)	39.58^{b} (4.55)	27.06^{ab} (5.38)
FOOD CONVERSION EFFICIENCY *	0.40^{a} (0.01)	0.45^{ab} (0.01)	0.61^{bc} (0.07)	0.65^{c} (0.05)	0.64^{c} (0.02)	0.66^{c} (0.03)	0.48^{abc} (0.03)
PROTEIN EFFICIENCY RATIO	1.22^{a} (0.05)	1.18^{a} (0.06)	1.19^{a} (0.13)	1.24^{a} (0.09)	1.28^{a} (0.12)	1.06^{a} (0.05)	0.93^{a} (0.14)
NITROGEN INTAKE (mg/day)	2.49^{a} (0.22)	3.15^{ab} (0.29)	4.34^{abc} (0.60)	4.65^{abc} (0.60)	5.13^{bc} (0.65)	6.02^{c} (0.76)	6.80^{c} (0.98)
CARCASS N. DEPOSITION (mg/day)	0.45	0.48	0.54	0.84	1.10	0.97	0.61
APPARENT N. UTILIZATION (%)	18.23	15.30	12.45	17.98	21.50	16.18	9.04
CARCASS COMPOSITION (% WET WT.BASIS)							
	INITIAL						
MOISTURE	78.15	75.43	76.60	75.21	73.78	73.63	73.80	75.45
CRUDE PROTEIN	14.75	15.52	15.03	14.80	15.76	16.00	15.77	15.14
LIPID	1.57	3.70	3.00	4.75	5.69	5.32	5.77	4.35
ASH	5.53	5.35	5.37	5.24	4.77	5.05	4.66	5.06
MORTALITY (%)		15.05	17.77	25.41	12.22	11.23	10.52	18.51

* Kruskal-Wallis analysis $p < 0.05$
Note. Values in the same row with same superscript are not significantly different ($p < 0.05$)

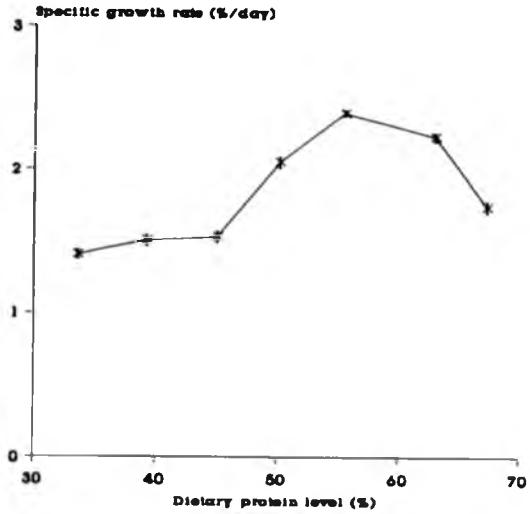


Figure 7. Specific growth rate of S.aurata fry against dietary protein level.

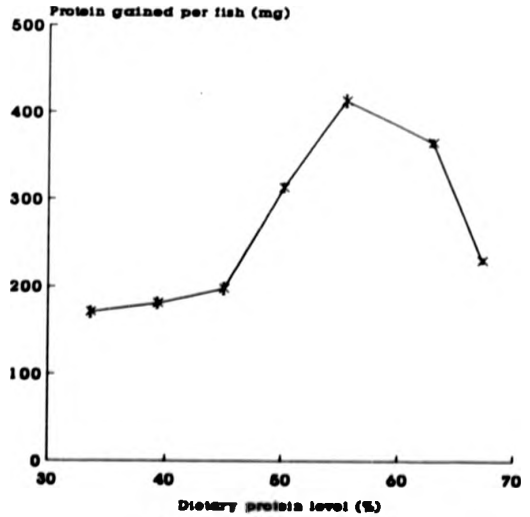
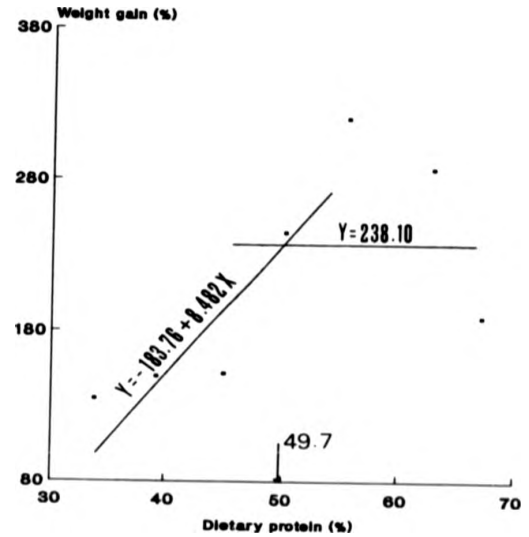


Figure 8. Protein gained in mg/ individual S.aurata fry at different dietary protein levels.

Protein gain per individual fish over the experimental period is shown in Figure 8. Again, there is a rapid increase with dietary protein level, with a maximum at 55.6% protein, followed by a decrease.

The data on percentage weight gain and mg protein gained per fish over the experimental period were re-plotted in Figure 9. These data were then analysed using the "broken-line" technique described by Zeitoun *et al.*(1976), in which the significantly different values at the lower protein levels are expressed by a linear regression and a second, horizontal line is derived from the mean values at the higher protein levels which are not significantly different from each other. The intersection of these lines is taken as an indication of the dietary protein requirement of the fish. In this case both figures suggest a protein requirement of 49.6% as an average (Figure 9).

The same data on percentage weight gain and mg protein gained per fish were analysed using a polynomial regression analysis also described by Zeitoun *et al.* (1976), in which a second order polynomial curve, represented by the equation $Y = B_o + B_1X + B_2X^2$, is characterized by having a unique maximum point (Y_{max}) along its range. The value of X_{max} that corresponds to Y_{max} is defined as an indication of the dietary protein that produces optimum growth or protein gain, and beyond which both parameters are depressed. In this case both figures suggest a protein requirement of 55.0% (Figure 10).



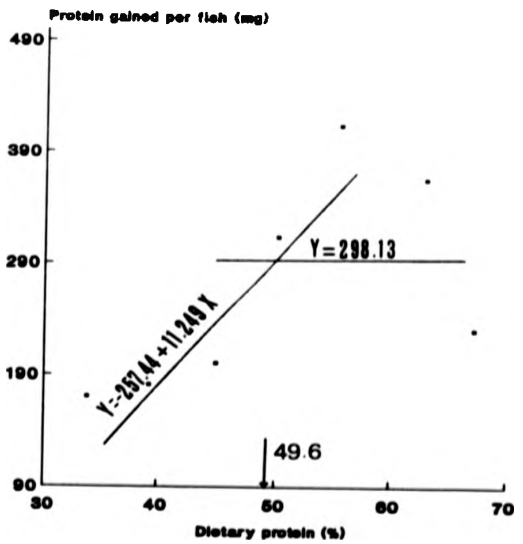
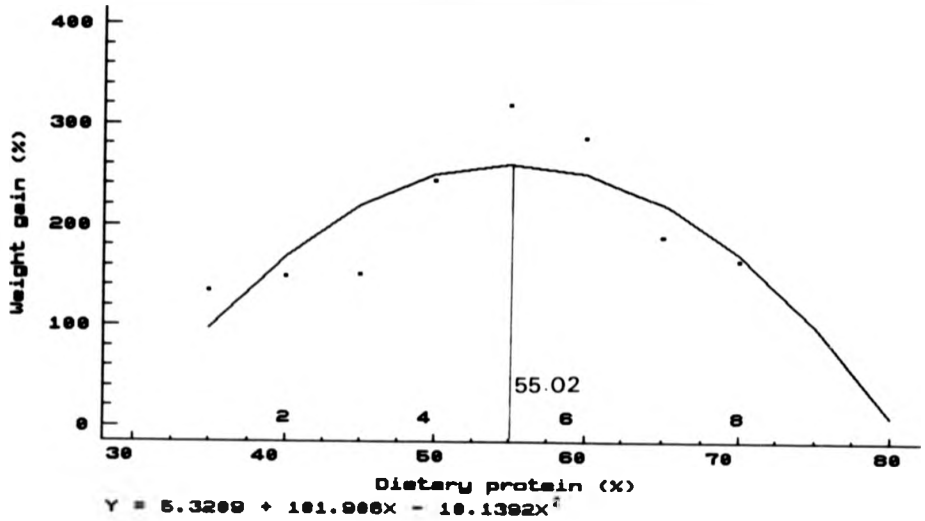


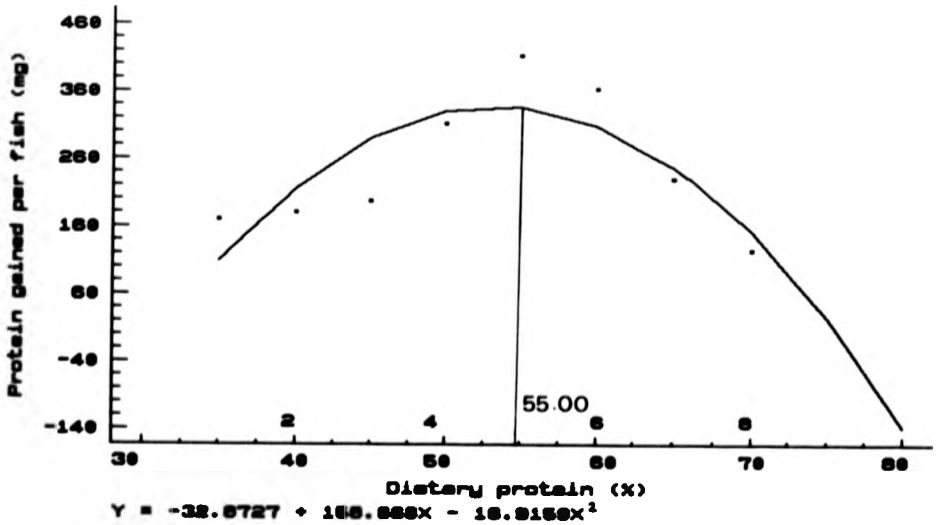
Figure 9. Broken line analysis for experiment with fry.

Figure 10.

Dose response analysis (polynomial regression) for experiment with fry.



$Y = 5.3209 + 101.906X - 10.1392X^2$



$Y = -32.8727 + 108.888X - 10.8158X^2$

3.3.1.2 Food Conversion Efficiency

There was a trend towards significantly ($p < 0.05$) better food conversion efficiencies as the dietary protein level increased. No significant differences ($p < 0.05$) were found in food conversion efficiency between the fish fed diets 4, 5 and 6 (50.1%, 55.6% and 63.0% protein, respectively). Beyond diet 6, which showed the highest FCE, diet 7 had a value similar to those supported by the lower protein diets (Table 12).

3.3.1.3 Protein Efficiency Ratio

After a slight decrease for diet 2 (39.3% protein), protein efficiency ratio (PER) values increased up to a maximum in diet 5 (55.6% protein), beyond which they showed a rapid decrease (diets 6 and 7). However, no significant differences ($p < 0.05$) were found between all diets (Table 12, Figure 11).

3.3.1.4 Apparent Net Nitrogen Utilization

Similarly to PER values, after a decrease from diets 1 to 3, the values of apparent net nitrogen utilization (APNU) increased to a maximum in diet 5 (55.6% protein), beyond which they showed a rapid decrease (diets 6 and 7)(Figure 11 and Table 12).

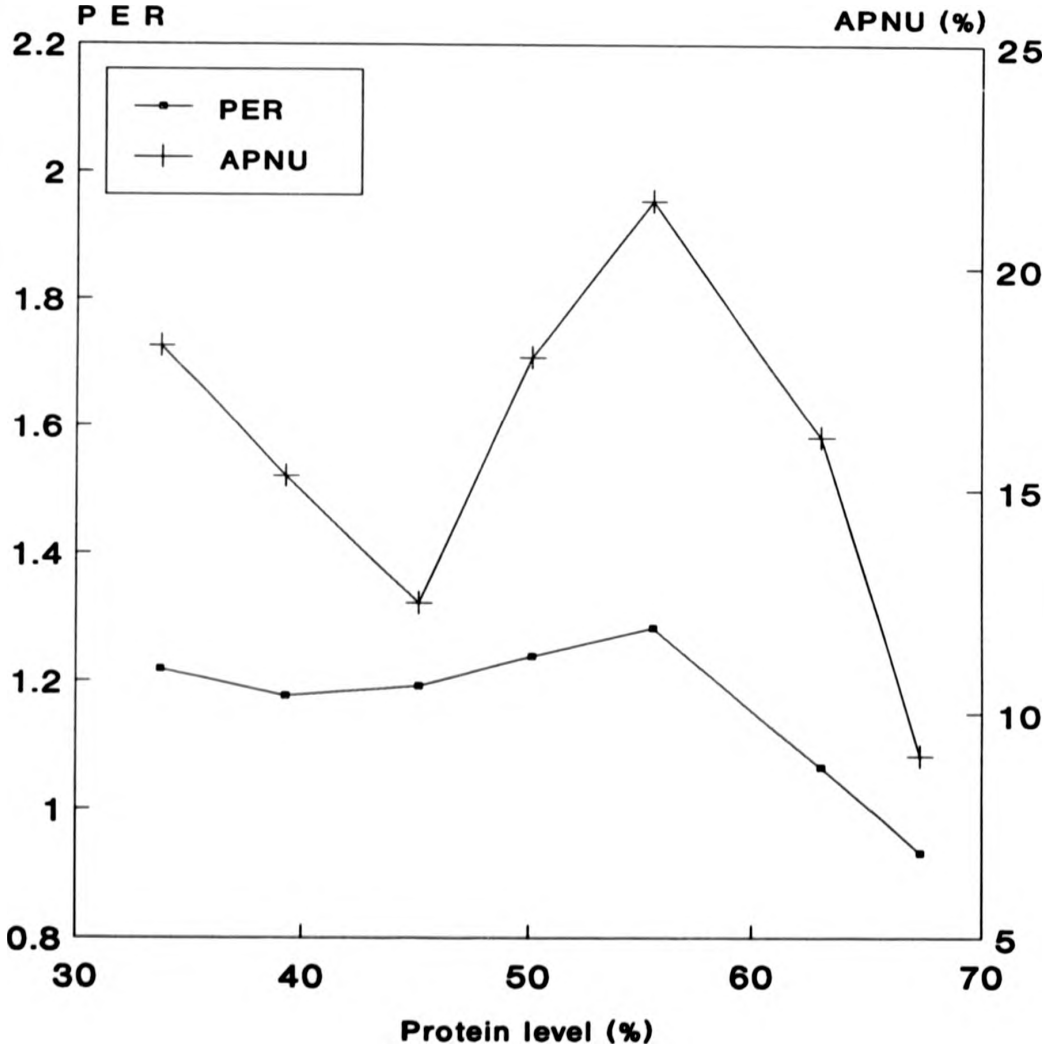


Figure 11. Protein efficiency ratio (PER) and apparent net nitrogen utilization (APNU) in experiment with fry.

3.3.1.5 Carcass Composition

The proximate composition of the whole fish carcass at the start and at the end of the experiment is shown in Table 12. In general, the body composition was not greatly affected by the administration of different levels of protein in the diets. The fish fed the higher protein levels tended to have lower moisture contents, higher lipid and protein than those fed the lower protein levels.

3.3.1.6 Mortality

Mortality varied from 10% to 18.5% (Table 12), with the exception of 25.4% mortality in diet 3, survivals were overall lower than expected. This may have been due to poor vigour of the fish batch used in the experiment, although these values can be considered within the normal range for this fish species and size.

3.3.2 EXPERIMENT II (Iuveniles)

3.3.2.1 Growth

The growth response of *S.aurata* juveniles over the experimental period at different protein levels is shown in Figure 12. The best growth response in

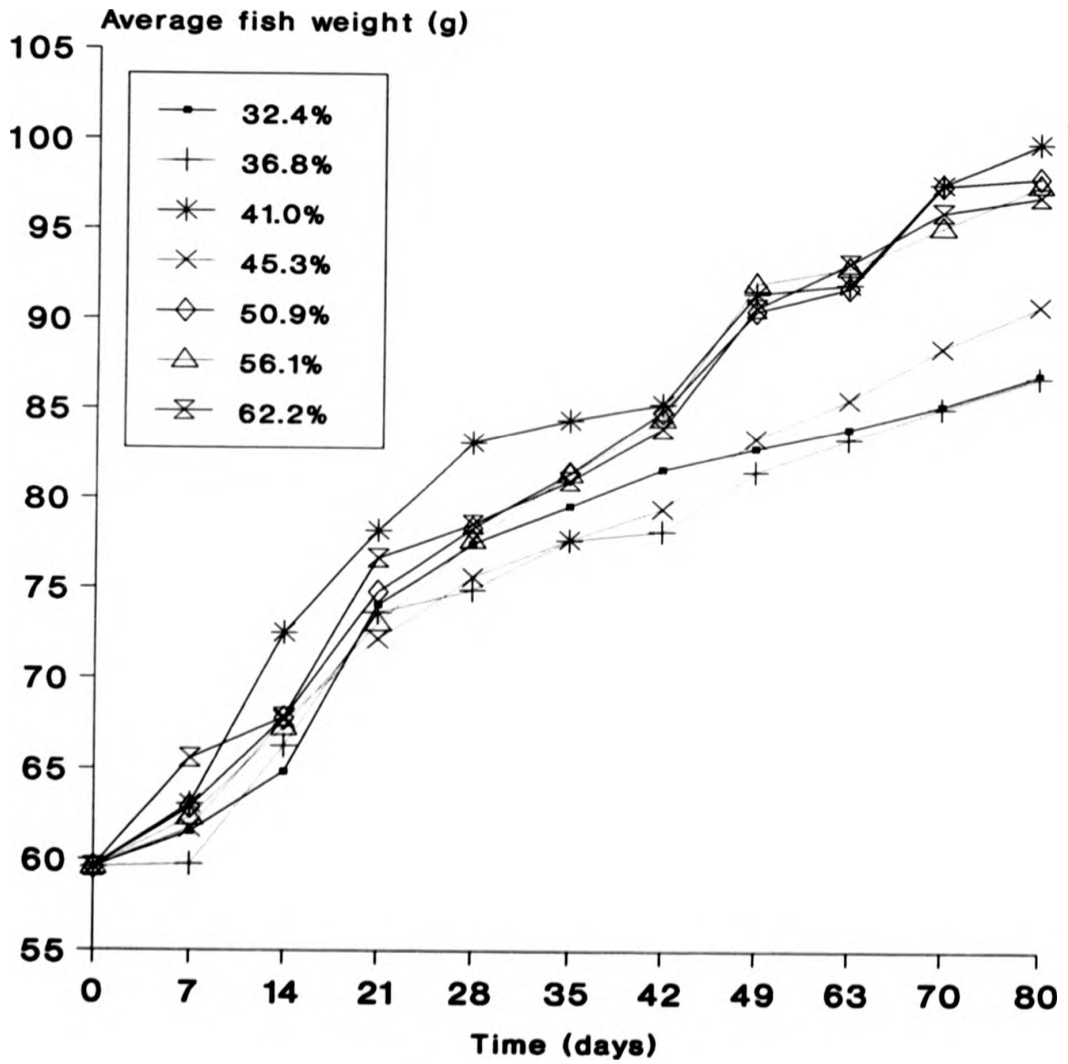


Figure 12. Growth response of S.aurata juveniles at different dietary protein levels.

terms of final body weight was obtained with diet 3 (41.0% protein), with a drecreasing trend between this protein level and the other diets following in the order: 5 (50.9% protein) > 6 (56.1% protein) > 7 (62.2% protein) > 4 (45.3% protein) > 1 (32.4% protein) > 2 (36.8% protein), diets 3, 5 and 2 being significantly different ($p < 0.05$) (Table 13).

A similar response was also observed on the basis of specific growth rate and percentage weight gain, the maximum value always being found for diet 3 (Table 13).

Figures 13 and 14 show specific growth rate and protein gain per individual fish over the experimental period. With the exception of diet 4 (45.3% protein), there seems to be a rapid increase of both parameters with dietary protein levels up to diet 3 (41.0% protein), beyond which there is a trend towards decrease.

The data on percentage weight gain and mg protein gained per fish over the experimental period were re-plotted in Figure 15. The data were then analysed using the "broken-line" technique described by Zeitoun *et al.* (1976), suggesting a protein requirement of 41.8% as an average.

The same data on percentage weight gain and mg protein gained per fish were analysed using the polynomial regression analysis described in experiment

Table 13
Mean growth performance, feed utilization efficiency and carcass composition of S.aurata juveniles fed the experimental diets.

MEAN VALUE RESULTS (Std. error in parenthesis)	DIET NUMBER						
PROTEIN IN FOOD (%)	1 32.4	2 36.8	3 41.0	4 45.3	5 50.9	6 56.1	7 62.2
INITIAL BODY WT.(g) (2.33)	59.6	59.6	59.6	59.6	59.6	59.6	59.6
FINAL BODY WT.(g)	87.09^{ab} (2.60)	86.92^{a} (1.65)	100.02^{c} (2.46)	90.92^{ab} (1.47)	98.08^{b} (3.15)	97.68^{ab} (1.16)	97.02^{ab} (3.69)
WEIGHT GAIN (%)	46.17^{ab} (4.37)	45.88^{a} (2.78)	67.87^{c} (4.14)	52.61^{ab} (2.46)	64.01^{b} (5.28)	63.94^{ab} (1.95)	62.84^{ab} (6.19)
SPECIFIC GROWTH RATE (%/day)	0.47^{a} (0.04)	0.47^{a} (0.03)	0.63^{a} (0.03)	0.53^{a} (0.02)	0.62^{a} (0.04)	0.62^{a} (0.02)	0.61^{a} (0.05)
FOOD FED (g/day)	0.98^{a} (0.09)	1.00^{a} (0.09)	1.16^{a} (0.11)	1.00^{a} (0.09)	1.07^{a} (0.08)	1.11^{a} (0.08)	1.08^{a} (0.11)
WEIGHT GAIN (g/day)	0.36^{a} (0.11)	0.34^{a} (0.08)	0.52^{a} (0.09)	0.38^{a} (0.07)	0.49^{a} (0.09)	0.47^{a} (0.08)	0.47^{a} (0.09)
FOOD CONVERSION EFFICIENCY	0.37^{a} (0.01)	0.34^{a} (0.02)	0.45^{b} (0.01)	0.38^{a} (0.001)	0.46^{b} (0.02)	0.42^{b} (0.01)	0.43^{b} (0.02)
PROTEIN EFFICIENCY RATIO	1.12^{a} (0.03)	0.92^{ab} (0.09)	0.91^{ab} (0.05)	0.84^{abc} (0.10)	0.90^{ab} (0.11)	0.75^{c} (0.07)	0.70^{c} (0.03)
NITROGEN INTAKE (mg/day)	51.20^{a} (2.17)	59.20^{b} (3.62)	75.84^{c} (1.93)	72.64^{c} (2.61)	87.36^{d} (3.33)	100.00^{e} (2.31)	107.04^{e} (1.73)
CARCASS N. DEPOSITION (mg/day)	9.60	9.55	13.78	11.78	14.48	14.81	12.76
APPARENT N. UTILIZATION (%)	18.75	16.13	18.17	16.22	16.57	14.81	11.92
CARCASS COMPOSITION (% WET WT.BASIS)							
	INITIAL						
MOISTURE	67.20	66.92^{a}	67.03^{a}	66.75^{a}	67.17^{a}	67.60^{ab}	67.88^{ab}	69.24^{b}
CRUDE PROTEIN	17.02	17.16^{a}	17.16^{a}	17.52^{ab}	17.63^{ab}	17.72^{ab}	17.96^{b}	17.03^{a}
LIPID	10.52	10.23^{a}	10.32^{a}	10.08^{a}	9.91^{a}	10.52^{a}	9.73^{a}	9.21^{a}
ASH	5.26	5.69^{a}	5.49^{a}	5.65^{a}	5.29^{ab}	4.16^{b}	4.43^{b}	4.52^{b}
MORTALITY (%)		6.67	6.67	13.33	8.89	8.89	28.89	13.33

Note. Values in te same row with same superscrit are not significantly different ($p < 0.05$)

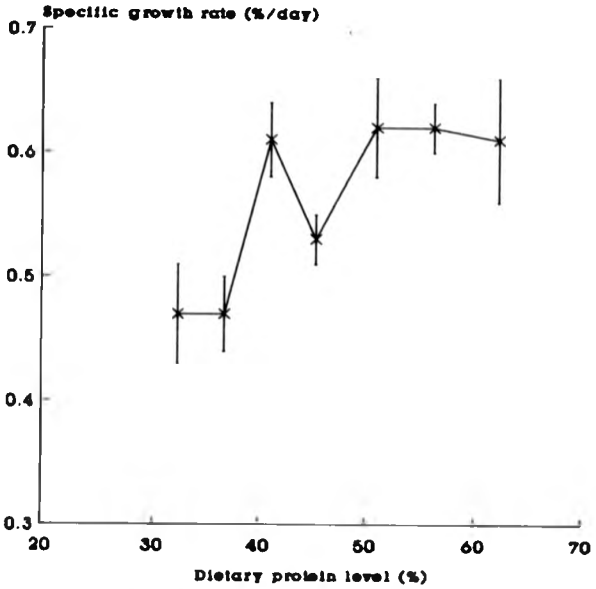


Figure 13. Specific growth rate of *S. aurata* juveniles against dietary protein level.

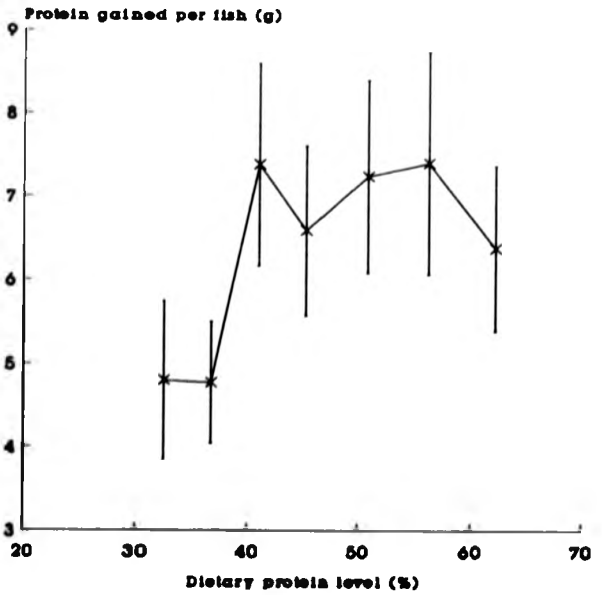
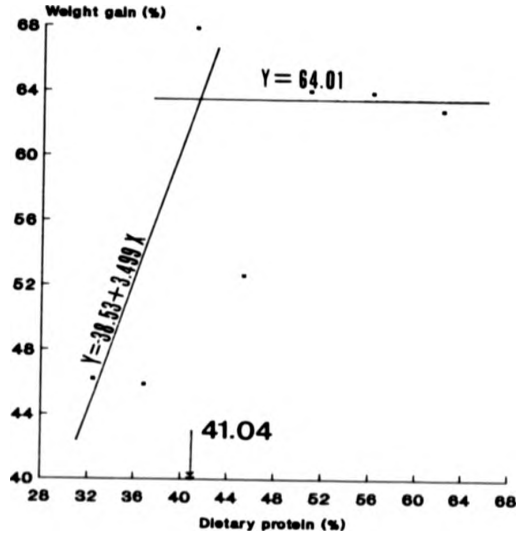


Figure 14. Protein gained in g/individual *S. aurata* juveniles at different dietary protein levels.



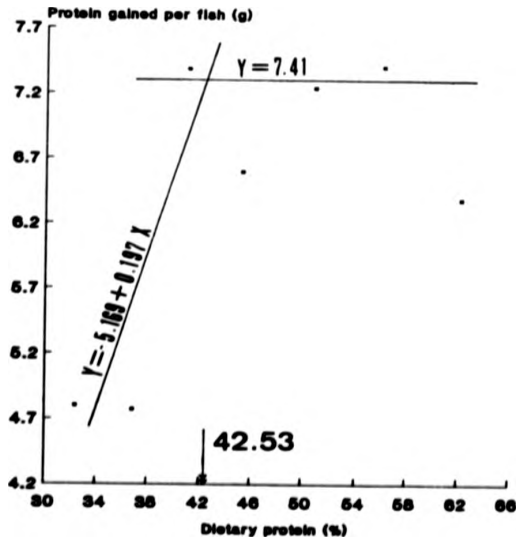


Figure 16. Broken line analysis in the juveniles experiment.

I, suggesting a protein requirement of 56.1% (% weight gain curve) and 50.9% (protein gained per fish curve)(Figure 16).

3.3.2.2 Food Conversion Efficiency

The best food conversion efficiency was observed with the fish fed diet 5 (50.9% protein), but with no significant difference ($p < 0.05$) from high protein diets 3, 6 and 7 (with the exception of diet 4). Lower protein diets (1 and 2) showed the poorest FCE, being significantly lower than those of high protein diets (Table 13).

3.3.2.3 Protein Efficiency Ratio

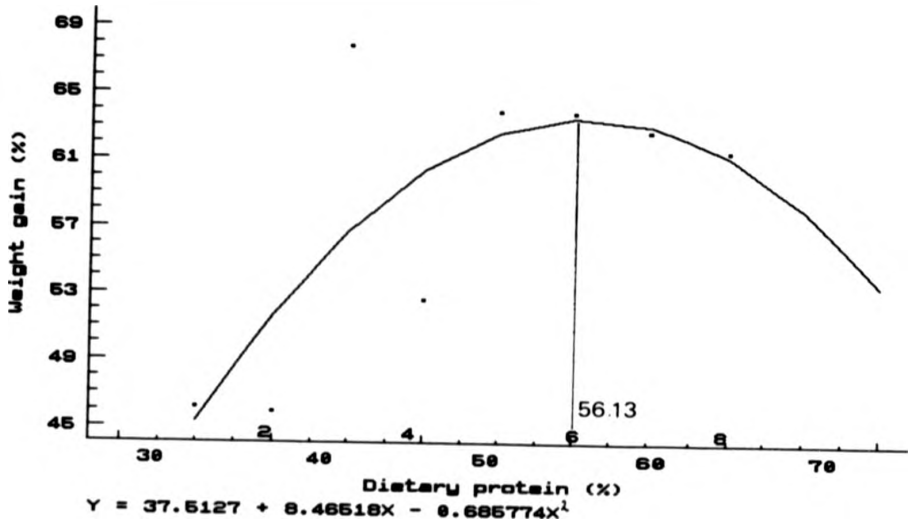
Protein efficiency ratio (PER) showed a trend towards reduction with increasing dietary protein. The highest PER was found for diet 1 (32.4% protein), the lowest value corresponding to diet 7 (62.2% protein), showing no significant difference with diet 6 (56.1% protein) (Table 13, Figure 17).

3.3.2.4 Apparent Net Nitrogen Utilization

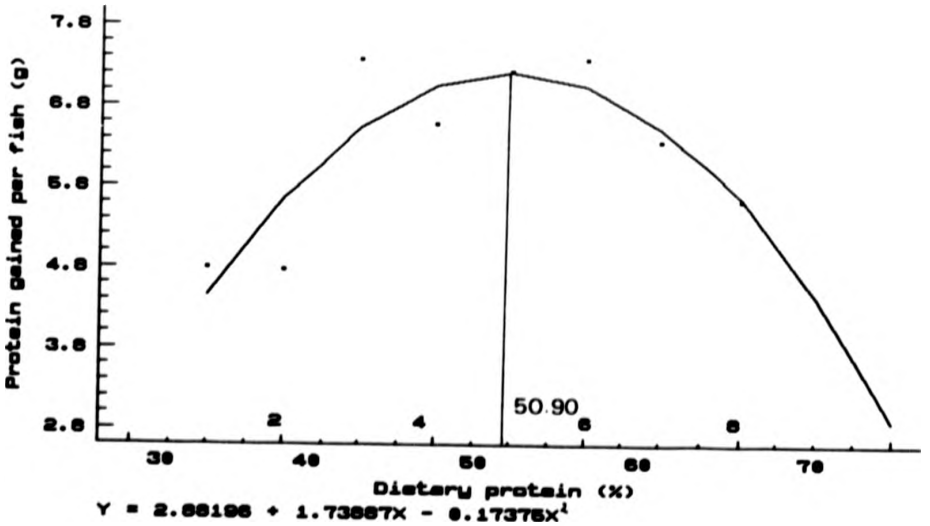
The values of apparent net nitrogen utilization (APNU), as well as PER, showed a trend towards decrease as dietary protein increased, the lowest value

Figure 16.

Dose response analysis (polynomial regression) in the experiment with juveniles.



$Y = 37.5127 + 8.46518X - 0.685774X^2$



$Y = 2.66196 + 1.73687X - 0.17376X^2$

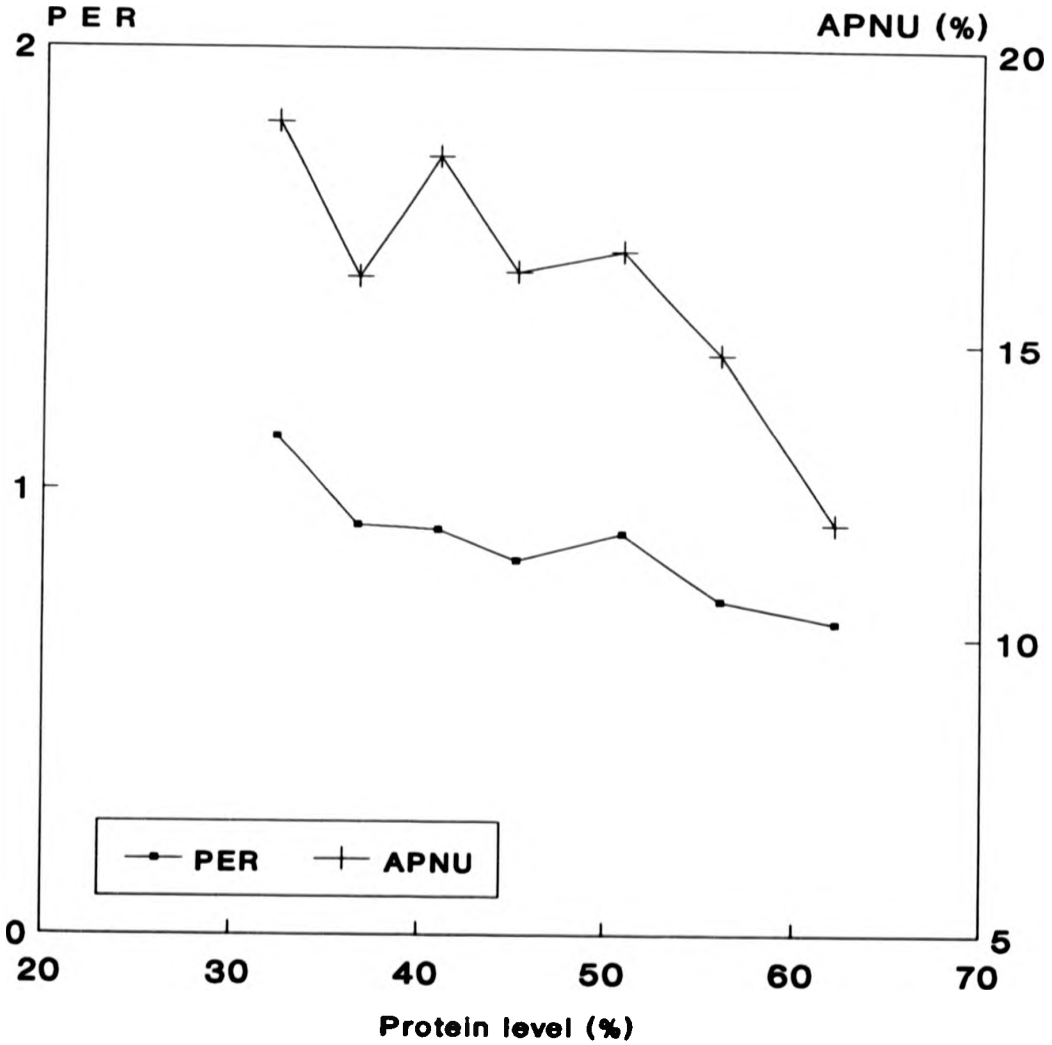


Figure 17. Protein efficiency ratio (PER) and apparent net nitrogen utilization (APNU) of S.aurata juveniles

being found in diet 7 with a dietary protein level of 62.2%, while the highest was in diet 1 (32.4% protein) (Table 13, Figure 17).

3.3.2.5 Carcass Composition

The proximate composition of the whole fish carcass at the start and at the end of the experiment is shown in Table 13. In general, the body composition was not greatly affected by the different treatments. The fish fed the higher protein levels tended to have lower lipid contents, higher moisture and protein than the lower protein levels.

The proximate composition of fish livers at the end of the experiment is shown in Table 14. In general, the fish fed the higher protein levels tend to have higher liver moisture and protein content, and lower carbohydrate content. Liver lipid content increased as dietary protein increased, but showed a decreasing trend beyond diet 4 (45.3% protein). Liver ash content decreased as dietary protein increased, but also increased beyond diet 4. There was a trend toward lower hepatosomatic indexes (HSI) with the increment of protein in diets (Table 14).

Table 14
Composition of fish livers at the end of the experiment (wet wt. basis)

DIET	1	2	3	4	5	6	7
MOISTURE	66.87	67.23	65.98	64.37	65.67	69.28	69.46
CRUDE PROTEIN	9.80	9.51	11.22	10.12	11.31	13.03	13.21
LIPID	6.25	6.18	10.06	13.13	10.48	7.05	6.57
ASH	3.92	1.86	1.04	0.85	0.91	0.92	0.99
NFE (1)	13.16	15.22	11.70	11.53	11.63	9.72	9.77
HSI (%) (2)	2.15	2.54	1.91	2.01	1.71	1.41	1.51

(1) NFE = Nitrogen free extractives, calculated as 100 - (% mois. + % prot. + % lip. + % ash)
(2) HSI = Hepatosomatic index, calculated as (wet liver wt./wet total body wt.) x 100

3.3.2.6 Mortality

Mortalities varied from 6.7% to 28.9%, being much lower than in experiment I. These values can be considered as normal for this species and fish size (Table 13).

3.4 DISCUSSION

When comparing figures 6 and 12 the minimum dietary protein supporting best growth of *S. aurata* in the present work seems to be influenced by the size of the fish, thus with fry the best growth was obtained with 56% dietary protein, while with juveniles the best growth was obtained with 41% dietary protein. These results agree with the well documented fact that smaller fish require higher levels of protein for maximal growth than larger fish. This effect is principally associated with a large decrease in whole body protein synthesis throughout development in fish, leading to a decrease in growth rate in older fish (Bilio *et al.*, 1979; NRC, 1983; Fauconneau, 1985; New, 1986; Steffens, 1989; Tacon, 1990).

The dose-response analysis of the data using the "broken-line" technique showed that the requirement of fry is 49.7% protein when the weight gain is used and 49.6% protein when the protein gain is plotted (Fig. 9). The same analysis applied to the data for juveniles gives values at 41.0% and 42.5% respectively (Fig. 15).

When the dose-response analysis was performed using the polynomial regression technique, the requirement of fry is 55.0% protein for weight gain and 55.0% protein for protein gain (Fig. 10). The same analysis on the data for juveniles gives values at 56.1% and 50.9% respectively (Fig. 16).

Thus, the protein requirement level obtained from the broken line technique appears significantly lower than that obtained from the polynomial regression analysis in both fish sizes and for percentage weight gain and protein gain.

Zeitoun *et al.* (1976) suggest that the polynomial regression analysis is the most accurate of these approaches, as it provides a better empirical fit to the growth responses of living organisms which do not exhibit an abrupt change from linearity as postulated in the broken-line analysis.

The same authors, however, state that by plotting confidence limits of 95% of all estimated responses of the curve, and by drawing a straight line parallel to the abscissa and passing through the maximum level of the lower line of the confidence limit, the intersections of this line with the left side of the polynomial curve and the upper line of the confidence limit will give dietary protein concentrations similar to those obtained from the broken line technique, which will still support adequate growth responses and minimize diet costs. In this way, the authors seem to minimize differences between the two techniques, concluding that both may lead to similar conclusions concerning dietary protein requirements.

However, a similar statistical approach could also be adapted to the broken line technique, and by plotting confidence limits of 95% of all estimated responses in the linear regression, lower dietary protein concentrations could also be obtained as optimum, which will maintain the difference from values obtained from the polynomial regression technique.

Thus, based on the above arguments, the dietary protein requirements obtained from the polynomial regression analysis could be considered as more biologically accurate. However, growth responses of *S.aurata* in this work seem to show a different fit to both analysis techniques in each of the two experiments.

In the experiment with fry, neither weight gain nor protein gain reached a plateau as dietary protein increased, providing a poor statistical basis for the use of the broken-line technique. In this case, the polynomial regression analysis seems to fit better to the fish responses, giving an optimum dietary protein level of 55%.

On the contrary, in the second experiment (juveniles), both weight and protein gains reached a plateau as dietary protein increased. In this case, the broken-line technique clearly provides a better empirical fit to the fish growth responses, giving an optimum dietary protein level of 42%.

However, neither of these clasical approaches was a particularly good fit

to the data generated. In fact the true situation appears to be more complex than either model suggests. Thus, in the experiment with fry, at least three different patterns appear both in weight gain and protein gain responses. A moderate increase from diets 1 to 3 is followed by a dramatic rise in diets 4 and 5, and finally diets 6 and 7 cause a marked decrease. In juveniles, an increase in dietary protein level in diets 1 and 2 provokes a moderate fall in the above responses, followed by a dramatic increase in diet 3 and reaching a plateau with the higher protein diets.

A possible biological reason for this could be the dual role of protein as "energy-yielding" and "tissue-yielding" nutrient, and the inter-relations with other dietary energy sources.

Both specific growth rate and food conversion efficiency increased for both fish sizes up to the apparent optimum protein requirement level and this was followed by a clear decrease when the protein was increased above that point. A similar response has been observed for *S. aurata* (Sabaut and Luquet, 1973), *Pleuronectes platessa* (Cowey *et al.*, 1972), *Mugil capito* (Papaparaskeva and Alexis, 1986), *Oncorhynchus mikiss* (Satia, 1974), *Channa micropeltes* (Wee and Tacon, 1982), *Morone saxatilis* (Millikin, 1982), *Micropterus dolomieu* and *M. salmoides* (Anderson *et al.*, 1981), *Epinephelus salmoides* (Teng *et al.*, 1978), *Oreochromis mossambicus* (Jauncey, 1982), and *Megalobrama amblycephala* (Shi *et al.*, 1988). This effect has been interpreted as due to diversion of energy required for growth toward deamination of excessive absorbed amino acids (Jauncey, 1982; Cho *et*

al., 1985).

The protein efficiency ratio (PER) in *S.aurata* fry increased as dietary protein rose up to the apparent optimum level (55%), then clearly dropped. Similar responses were reported for *Pleuronectes platessa* (Cowey *et al.*, 1972), *Epinephelus salmoides* (Teng *et al.*, 1978), and *Channa micropeltes* (Wee and Tacon, 1982). Apparent net nitrogen utilization (APNU) reached its optimum at 55.5% protein and then decreased also in fry. A similar decrease beyond optimum level is been reported for *Cyprinus carpio* (Ogino and Saito, 1970), *Fugus rubripes* (Kanazawa *et al.*, 1980), *Micropterus dolomieu* and *M.salmoides* (Anderson *et al.*, 1981), *Ctenopharyngodon idella* (Dabrowsky, 1977). Thus, low protein utilization was taking place at the lowest dietary protein levels; as the protein level increased, utilization increased and reached a maximum near the minimum requirement. As protein levels passed beyond the minimum requirement, utilization decreased.

Both PER and APNU in *S. aurata* juveniles showed a clear reduction as the dietary protein level increased. Similar observations were described in *Anguilla anguilla* (Higuera *et al.*, 1989), *S. aurata* (Sabaut and Luquet, 1973), *Oncorhynchus mykiss* (Satia, 1974), *Anguilla japonica* (Nose and Arai, 1972) and *Oreochromis mossambicus* (Jauncey, 1982).

The liver composition of *S. aurata* juveniles at the end of the experiment showed that a higher moisture and protein content, and a lower carbohydrate

and ash content, were apparent as dietary protein level increased, suggesting increased protein synthesis as similarly reported for *Mugil capito* (Papaparaskeva and Alexis, 1986). A certain increase in liver lipid level was also showed for the higher protein diets, as also reported for *Morone saxatilis* (Millikin, 1982).

The hepatosomatic index (HSI) of juveniles showed a trend towards decrease as dietary protein increased and similar to changes in liver carbohydrate content, probably related to higher carbohydrate content in low protein diets, leading to liver glycogen accumulation. Similar effects have also been reported for *Anguilla japonica* (Nose and Arai, 1972), *Pleuronectes platessa* (Cowey *et al.*, 1975), *Morone saxatilis* (Millikin, 1982), *Channa micropeltes* (Wee and Tacon, 1982) and *Dicentrarchus labrax* (Metailler *et al.*, 1981).

The mortality of the fish during both trials was not affected by protein level in the diets.

These results, together with the dose-response analysis, indicate that the optimum protein levels in the diet for *S.aurata* fry and juveniles for maximum protein retention and optimum growth are around 55% and 42%, respectively, and are in the same range as other marine carnivorous fish species such as *Pleuronectes platessa* (50%), *Chrysophrys major* (54%), *Seriola quinqueradiata* (55%), *Epinephelus salmoides* (40-50%) and *Fugus rubripes* (50%) shown in Table 2.

The optimum dietary protein level for fry (55%) is significantly higher

than 40% protein found optimum for 3g *S.aurata* fingerlings by Sabaut and Luquet (1973), who used casein supplemented with a mixture of synthetic amino acids as dietary protein source. This fact, as explained in the introduction (Section 3.1) could have depressed growth and feed efficiency in fish. The use by these authors of non-isoenergetic diets and "ad libitum" feeding regimes could have favoured the use of protein in higher protein diets for energy purposes in a higher proportion than in lower protein diets (Higuera, 1987).

These experimental differences, together with different environmental conditions may, at least partially, explain different results obtained.

Comparisons between experiments on nutrient requirements are sometimes impossible due to the great variation of techniques and protocols utilized by different authors. In these experiments, although calculated gross and metabolizable energy content indicate that in both experiments diets were isoenergetic, the use of empirical values may have lead to diminished accuracy, and the fixed feeding regime, although not restrictive, may have favoured the growth of those fish fed the higher protein diets.

These results, as well as the lack of apparent strong differences between the protein requirements of vegetarian, carnivorous and ichthyophagous fish, quite often argued (Martinez-Palacios, 1987; Cowey, 1975), underlines the need for more research, as well as standardization of experimental methods in order to obtain comparable results (Gropp and Tiews, 1981).

4. STUDIES ON THE PROTEIN-SPARING EFFECT OF DIETARY LIPID IN *Sparus aurata* AT TWO DIFFERENT SIZES

4.1 INTRODUCTION

Among those factors influencing quantitative dietary protein requirements for a particular fish species is the amount of non-protein energy in the diet, as some of the dietary protein may be utilized as an energy source (Cho *et al.*, 1985).

Ideally, the energy derived from protein catabolism should be provided from other nutrients (i.e., lipids, carbohydrates) in order to maximise the level of protein used for anabolic processes (Cowey, 1979; Steffens, 1989).

Dietary lipids serve both as sources of essential fatty acids (EFAs) and energy. In addition, they also act as carriers of fat-soluble vitamins (New, 1986). Lipids play an important role as energy sources in fish diets, especially for carnivorous fish in which the availability of dietary carbohydrates for energy is limited. Poor carbohydrate digestibility in these fish species is due to a limited amylolytic activity in their digestive tract (Spanhof and Plantikow, 1983); other metabolic constraints seem to be a limited regulation of blood glucose levels (Bergot, 1971; Palmer and Ryman, 1972), and a scarce use of liver glycogen reserves (Chang and Idler, 1960; Dave *et al.*, 1975). Thus, main sources of energy in fish appear to be protein and lipids, carbohydrates being considered as uncertain and limited (Walton, 1987). The protein requirements of fish may be reduced by increasing the level of dietary, non-protein, nutrients by virtue of their "energy-yielding" properties ("Protein sparing effect") (Jauncey and Ross,

1982). The dynamics of fat and protein accumulation and utilization in fish are, however, difficult to interpret, mainly because these relationships are largely influenced by a number of factors, such as age of fish, migratory behaviour, gonad maturation, feeding ratio, etc. (Weatherley, 1976)

It is broadly agreed that addition of fats in fish diets contributes to the protein-sparing effect by increasing the digestible energy of the diets. However, food intake is controlled by the dietary energy level, and if the diet contains an excess of non-protein energy, appetite or demand may be satisfied before a sufficient quantity of protein (and possibly other nutrients) is ingested to satisfy demand for maximal rates of protein synthesis and growth (Bromley, 1980; Metailler *et al.*, 1981; Alsted and Jokumsen, 1989). At the same time an elevated dietary fat content frequently stimulates a high fat carcass deposition, which may not always be desirable. On the other hand, if the diet is deficient in non-protein energy, protein will be used for energetic purposes (basal metabolism and voluntary activity) rather than for protein synthesis (Cowey *et al.*, 1975; Alliot *et al.*, 1979; Cho, 1987).

Thus, the design of formulated diets is a compromise between a protein content that will allow good growth with least possible conversion to energy and an energy content leading to high rates of protein synthesis but not such as to produce excessive body lipid deposition (Weatherley and Gill, 1987).

One of the most important factors affecting the optimum protein and lipid level in a fish diet is probably temperature (New, 1986). Cho *et al.*(1985) suggest that dietary protein:energy ratio should be altered to meet changes in environmental temperatures, particularly as fish appear to be well adapted to deal with temperature changes within their thermal tolerance limits.

Marais and Kissil (1979) reported that low energy diets containing 43% protein and 9% lipid (P:E ratio: 22 mg/KJ), supported better growth performance of 44g *S.aurata* juveniles than high energy diets (43% protein and 16% lipid; P:E ratio: 20 mg/KJ). Experimental diets contained a mixture of fish meal (22%), meat meal (15%) and soya meal (63%) as protein source, and soya oil as main source of dietary lipids.

Kissil and Groop (1984) found that the best P:E ratio for 45g *S. aurata* juveniles was 25 mg/KJ (40/5 = %prot/%lip), and 23 mg/kJ (44/10 = %prot/%lip) for 3g fingerlings, the diets containing a mixture of fish meal, meat and poultry meal, soy bean meal, feather meal, fish concentrate protein and soy bean protein as protein source, and capelin fish oil as the main source of non-protein energy.

On the other hand, Pereira *et al.*(1987) reported that high protein, high energy diets (51/14 = %prot/%lip) supported better growth performance in 1-6g *S.aurata* when diets with a range of 46 to 51% dietary protein and a fixed 14% lipid content were tested. Fish meal and cod liver oil were the main sources of dietary protein and lipid, respectively.

Takeuchi *et al.*(1991) found that the optimum combination of dietary protein and lipid for 2-7g red sea bream was 52% and 15%, respectively, when white fish meal plus casein and pollack liver oil were used as sources of dietary protein and lipid, respectively. Dietary protein ranged from 38 to 55%, and dietary lipid from 5 to 21%.

The aim of the present study was to determine the optimum protein to energy ratio for *S.aurata* fingerlings and growers for the particular environmental conditions of the Canary Islands, especially with regard to temperature. Sardine meal was used as the dietary protein source and sardine oil as the main non-protein source of dietary energy.

4.2 MATERIALS AND METHODS

For these two trials, III and IV, sea bream fingerlings and growers of mean body weights 5.3g and 89.8g were employed, respectively. Experimental fish were stocked at 20 fingerlings per tank and 30 growers per tank, three tanks per treatment.

Prior to starting the experiments, both fingerlings and growers were acclimated for one week, feeding them with diets containing 51.8/12.1 and 52.6/6.4 (%prot/%lip) at rates of 3% and 2% of body weight per day, respectively. At the start of the experiments 10 fingerlings and 10 growers were

killed by an overdose of benzocaine and stored at -20°C for subsequent chemical analysis.

Tank system I (Section 2.2.1) was utilized for the fingerlings trial, and tank system II (Section 2.2.2) for the growers experiment. Table 15 shows the mean values obtained for the environmental parameters, as detailed in Section 2.3), recorded during the two experiments. All values were within the optimum ranges for this species (Section 1.6).

Eight experimental diets fed to gilthead sea bream fingerlings were prepared with varying amounts of the same ingredients in such a way that four dietary protein levels, 40%, 45%, 50% and 60%, were combined with two dietary lipid levels, 9% and 15%. In the second experiment, two dietary protein levels (45% and 53%) were combined with two dietary lipid levels (7% and 12%) to produce four experimental diets.

Dietary protein in all experimental diets was derived from sardine meal, which also provided some lipid. Protein content was decreased by replacing fish meal with dextrin and corn starch in a proportion of 1:3 in the fingerlings trial. Wheat bran was used in the experiment with growers to replace dietary protein, as agreed with the fish meal producers (AGRAMAR, A/S), who funded this experiment. α-cellulose was used as filler and carboxy-methyl cellulose as binder in both experiments.

Table 15
Environmental parameters recorded during protein-energy experiments with fingerlings and growers.

FINGERLINGS	
Temperature	21.77°C ± 0.47
pH	8.12 ± 0.01
Dissolved oxygen	6.65 ± 0.55 mg/l
Salinity	36.6 ± 0.02 ppt
Nitrite	0.009 ± 0.003 mg/l
GROWERS	
Temperature	21.20°C ± 1.37
pH	8.12 ± 0.02
Dissolved oxygen	6.65 ± 0.25 mg/l
Salinity	36.6 ± 0.02 ppt
Nitrite	0.007 ± 0.002 mg/l

In both trials, the experimental diets were designed to obtain approximately isoenergetic diets for each protein level in order to study the different combinations of both protein and lipid levels in the diets. Different P:E ratios were achieved in both experiments by using different protein levels.

The diameters of dry pellets were 1mm and 3mm for fingerlings and growers, respectively (Length:diameter ratio: 2:1). Tables 16 and 17 show the composition of the experimental diets used with fingerlings and growers, respectively.

The feeding regimes used throughout the trials were 3% and 2% body weight per day, respectively. The frequency of feeding was maintained at 5 times a day, 6 days a week, and twice on sundays. The trials were conducted for 57 days with fingerlings and 84 days with growers.

At the start of the experiments and at subsequent fortnightly intervals, fish were individually weighed to the nearest one decimal place (0.1g) on a Mettler top-pan balance (Model PE-3000). Bulk weighings were practically impossible to perform with these fish sizes but would have otherwise been preferable due to less stressful handling and anaesthetizing.

At the end of the experiments, fish were killed by an overdose of benzocaine, then individually weighed, and half the population in every tank

Table 16
Composition of the experimental diets used in the experiment with sea bream fingerlings.
Proximate analysis on a moisture free basis, with the calculated energy and protein:energy ratio.

INGREDIENTS (%)	DIET NUMBER							
	1	2	3	4	5	6	7	8
Sardine meal(1)	58.00	58.00	66.20	66.20	74.40	74.40	82.50	82.50
Corn dextrin	8.30	5.14	6.59	3.43	4.87	1.71	3.16	0.00
Corn starch	24.91	15.44	19.76	10.29	14.62	5.15	9.47	0.00
Sardine oil	2.63	8.63	1.73	7.73	0.82	6.82	0.00	6.00
α-Cellulose	1.66	8.29	1.22	7.85	0.79	7.42	0.37	7.00
C.M.Cellulose(2)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix(3)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix(3)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
NUTRIENT CONTENT (%)								
Crude protein	40.84	44.46	47.82	46.50	53.09	51.81	59.01	58.17
Ether extract	8.86	14.87	8.83	14.84	8.81	14.87	8.90	14.89
Ash	13.02	12.96	13.86	13.29	15.12	14.96	16.46	16.28
Fibre	1.22	6.34	1.00	6.73	1.16	7.14	0.20	6.42
NFE (4)	37.28	27.71	29.49	25.37	22.98	18.36	15.63	10.66
GE (MJ/Kg)(5)	19.55	21.13	19.84	21.20	19.96	21.26	20.13	21.44
ME (MJ/Kg)(6)	16.31	17.73	16.47	17.77	16.51	17.75	16.58	17.83
P:E ratio (7)	20.89	21.04	24.10	21.93	26.60	24.37	29.31	27.13

(1) Proximate analysis (% dry wt.): Crude protein: 69.6, Ether extract: 11.6, Ash: 18.2.
(2) Carboxy methyl cellulose (Sodium salt)
(3) Table 7, Section 2.4.
(4) NFE = Nitrogen free extractives, calculated as 100 -(% prot. + % lip. + % ash).
(5) GE = Gross energy content.
(6) ME = Metabilizable energy content.
(7) P:E = Protein to energy ratio in g protein/Mj of GE.

Table 17
Composition of the experimental diets used in the experiment with sea bream growers.
Proximate analysis on a moisture free basis, with the calculated energy and protein:energy ratio.

INGREDIENTS (%)	DIET NUMBER			
	1	2	3	4
Sardine meal (1)	51.02	51.02	68.03	68.03
Wheat bran (2)	45.08	40.08	29.07	24.07
Sardine oil	1.00	6.00	0.00	5.00
C.M.Cellulose (3)	0.50	0.50	0.50	0.50
Vitamin premix (4)	2.00	2.00	2.00	2.00
Mineral premix (4)	0.40	0.40	0.40	0.40
NUTRIENT CONTENT (%)				
Crude protein	46.1	44.4	52.6	54.2
Ether extract	7.9	11.7	6.4	11.3
Ash	11.9	11.9	13.4	15.3
Fibre	4.6	5.6	3.6	2.7
NFE (5)	37.0	31.9	27.7	19.1
GE (MJ/Kg)(6)	19.65	20.61	19.68	20.55
ME (MJ/Kg)(7)	16.35	17.23	16.22	17.05
P:E ratio (8)	21.95	21.53	26.72	26.38

(1) Proximate analysis (% dry wt.): Crude protein: 62.4, Ether extract: 8.34, Fibre: 9.94, Ash: 17.37.
(2) Proximate analysis (% dry wt.): Crude protein: 16.9, Ether extract: 4.0, Fibre: 16.3, Ash: 5.5.
(3) Carboxy methyl cellulose (Sodium salt).
(4) Table 8, Section 2.4.
(5) NFE = Nitrogen free extractives, calculated as 100 - (% prot. + % lip. + % ash).
(6) GE = Gross energy content.
(7) ME = Metabolizable energy content.
(8) P:E = Protein to energy ratio in g protein/Mj of GE.

was eviscerated and the liver weights recorded against their respective whole carcass weights. Both whole and eviscerated carcass plus livers were then dried to obtain total moisture and later they were finely ground for chemical analysis.

Fish mortality was recorded daily and the weights of dead fish were discounted from the total in order to adjust the daily amount of food offered.

4.3 RESULTS

4.3.1 EXPERIMENT III (Fingerlings)

4.3.1.1 Growth

The growth response of *S. aurata* fingerlings over the experimental period under different protein to energy ratios is shown in Figure 18. The best response in terms of final body weight was obtained with diet 6 (51.8/14.9 = %prot/%lip), although no significant difference ($p < 0.05$) was found between this prot/lip level and diets 4, 7 and 8 (46.5/14.8, 59.0/8.9 and 58.2/14.9 = %prot/%lip, respectively).

Diet 1 gave the poorest results, being statistically different ($p < 0.05$) from all other diets (Table 18).

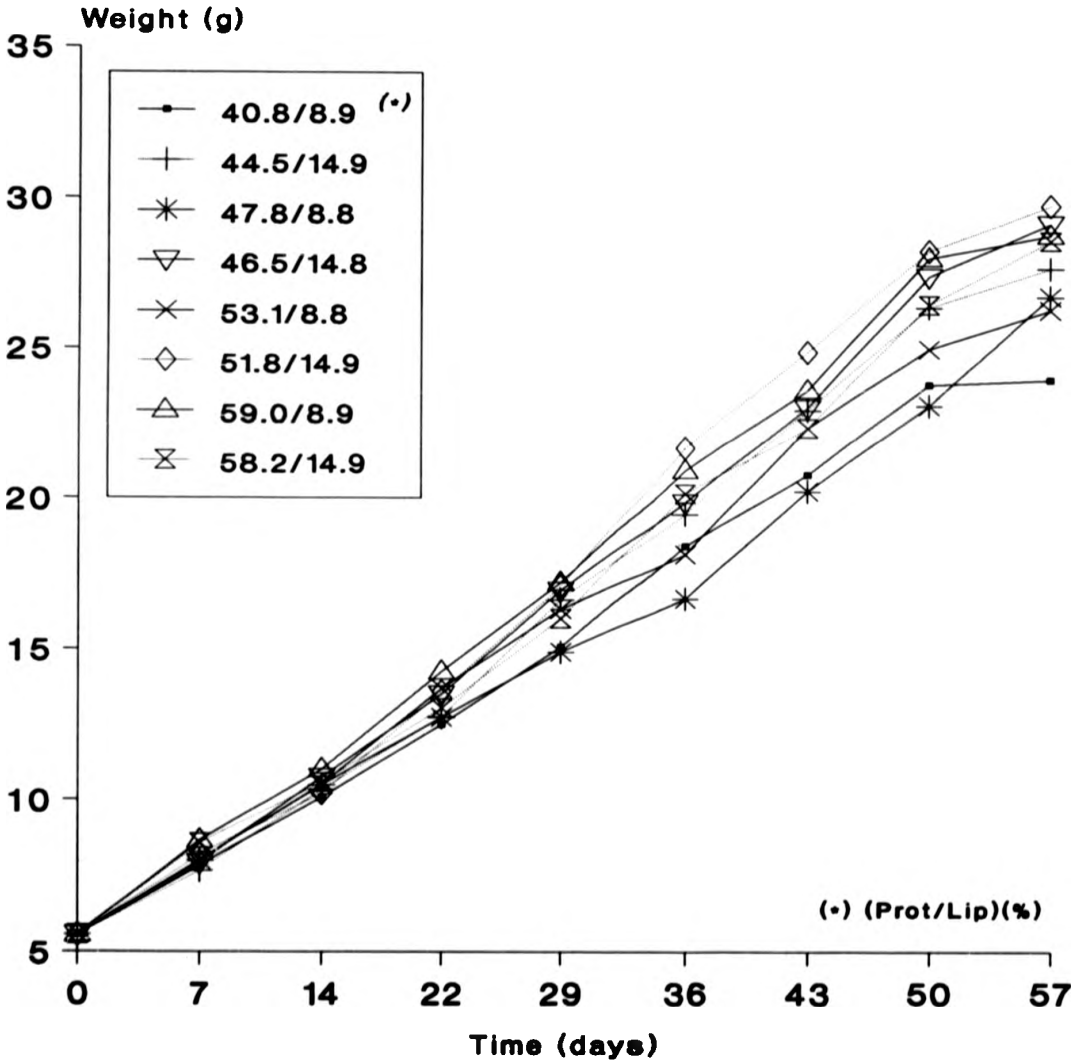


Figure 18. Growth response of S.aurata fingerlings at different dietary protein and lipid levels.

Table 18
Mean growth performance, feed utilization efficiency and carcass composition of S.aurata fingerlings fed the experimental diets.

MEAN VALUE RESULTS (Std. error in parenthesis)	DIET NUMBER							
(%) (Prot/Lip)	1 (40.8/8.9)	2 (44.5/14.9)	3 (47.8/8.8)	4 (46.5/14.8)	5 (53.1/8.8)	6 (51.8/14.9)	7 (59.0/8.9)	8 (58.2/14.9)
INITIAL BODY WT(g) (0.5)	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
FINAL BODY WT.(g)	24.0^{a} (0.7)	27.7^{ac} (0.9)	26.8^{abc} (0.7)	29.2^{c} (0.8)	26.3^{abc} (0.8)	29.8^{c} (0.9)	28.8^{c} (0.9)	28.7^{c} (0.9)
WEIGHT GAIN (%)	330.9^{a} (12.9)	397.8^{ac} (16.2)	389.5^{ab} (11.9)	425.0^{c} (14.1)	372.9^{abc} (15.1)	435.8^{c} (16.2)	417.9^{c} (17.1)	414.7^{c} (15.9)
SGR (%/day)(1)	2.5^{a} (0.05)	2.8^{ab} (0.06)	2.8^{ab} (0.05)	2.9^{b} (0.04)	2.7^{ab} (0.05)	2.9^{b} (0.05)	2.8^{b} (0.06)	2.8^{b} (0.06)
FOOD FED (g/day)	0.52^{a} (0.05)	0.56^{a} (0.05)	0.54^{a} (0.04)	0.57^{a} (0.05)	0.52^{a} (0.04)	0.62^{a} (0.06)	0.57^{a} (0.05)	0.59^{a} (0.05)
WEIGHT GAIN(g/day)	0.37^{a} (0.02)	0.43^{a} (0.03)	0.40^{a} (0.03)	0.45^{a} (0.03)	0.42^{a} (0.03)	0.46^{a} (0.04)	0.46^{a} (0.02)	0.46^{a} (0.04)
FCE (2)	0.71^{a} (0.03)	0.77^{a} (0.05)	0.75^{a} (0.03)	0.79^{a} (0.06)	0.81^{a} (0.02)	0.74^{a} (0.05)	0.81^{a} (0.03)	0.78^{a} (0.04)
PER (3)	2.06^{a} (0.19)	2.35^{a} (0.34)	1.96^{a} (0.26)	2.21^{a} (0.28)	1.91^{a} (0.24)	1.85^{a} (0.27)	1.85^{a} (0.27)	1.66^{a} (0.18)
APNU (%)(4)	28.11^{a}	26.79^{ab}	24.53^{c}	28.30^{a}	26.29^{b}	23.62^{c}	23.55^{c}	21.20^{d}
APLU (%)(5)	76.61^{d}	57.47^{b}	72.65^{c}	62.59^{bc}	82.74^{e}	52.03^{a}	73.16^{c}	56.06^{b}
CARCASS COMPOSITION (% WET WT.BASIS)								
	INITIAL							
MOISTURE	73.9	68.90^{a}	69.06^{a}	69.07^{a}	67.89^{a}	69.62^{a}	68.56^{a}	70.19^{a}	68.20^{a}
PROTEIN	15.73	16.75^{a}	15.92^{b}	17.07^{a}	16.55^{ab}	16.98^{a}	17.10^{a}	17.07^{a}	16.99^{a}
LIPID	5.68	9.64^{b}	10.93^{c}	9.21^{ab}	11.30^{c}	9.42^{ab}	10.14^{bc}	8.46^{a}	10.85^{c}
ASH	4.69	4.71	4.09	4.65	4.26	3.98	4.20	4.28	3.96
MORTALITY (%)		10.33	6.67	6.67	1.67	11.67	5.00	6.67	1.67

(1)SGR = Specific growth rate, (2)FCE = Food conversion efficiency, (3)PER = Protein efficiency ratio, (4)APNU = Apparent nitrogen utilization, (5)APLU = Apparent lipid utilization.
Note. Values in the same row with same superscript are not significantly different ($p < 0.05$).

These results suggest that, for a given protein level, fish grew better when dietary lipid level was increased from 8.9% to 14.9%, with the exception of diets containing 58-59% dietary protein, where growth values did not differ significantly ($p < 0.05$) as dietary lipid increased. Figure 19 shows the growth response of fish when high energy diets are compared to low energy ones.

The increase of dietary protein level also enhanced fish growth up to diet 6 (51.8% protein), beyond which growth response was depressed (Figure 19).

A similar response was also observed on the basis of percentage weight gain and specific growth rate, the maximum value always being found for diet 6. This, again, was not significantly different ($p < 0.05$) from diets 4, 7 and 8 (Table 18, Figure 20).

4.3.1.2 Food Conversion Efficiency

There was a trend towards an increase in food conversion efficiency as dietary lipid increased for diets containing protein up to 47% (Diets 3 and 4). Beyond this level, FCE decreased as dietary lipid increased. In all cases, values did not show a significant difference ($p < 0.05$) between them (Table 18, Figure 20).

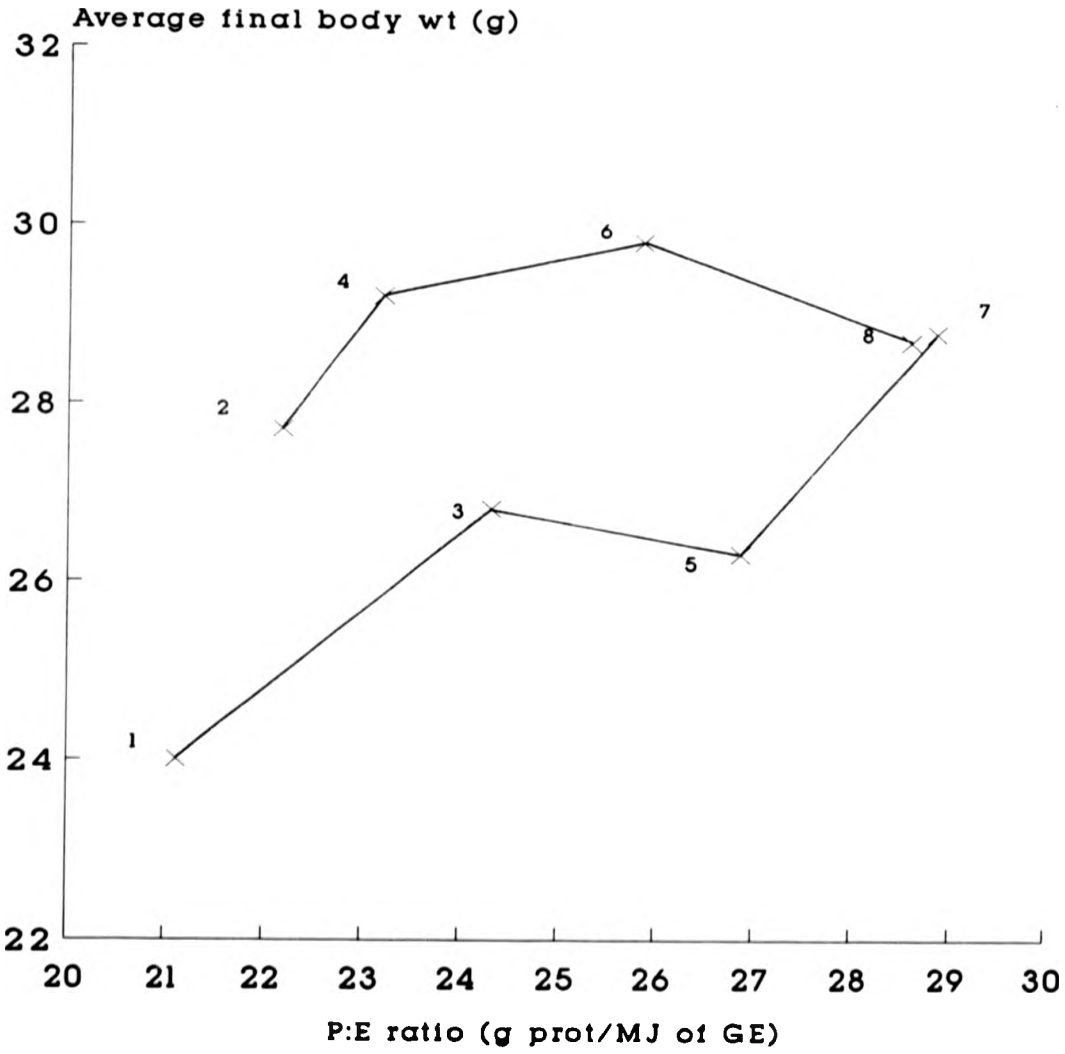
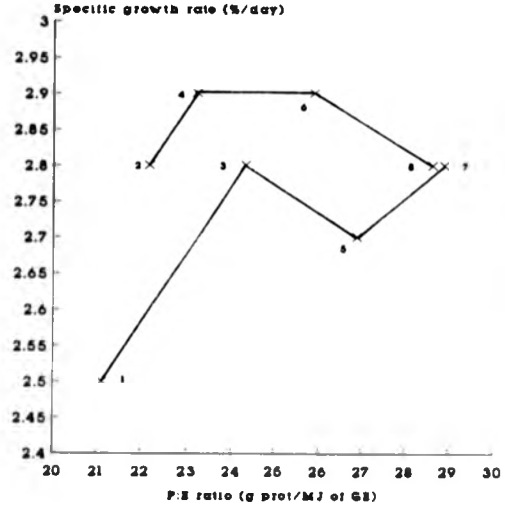
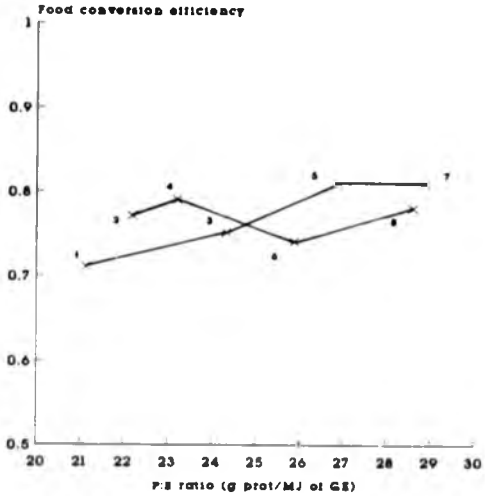


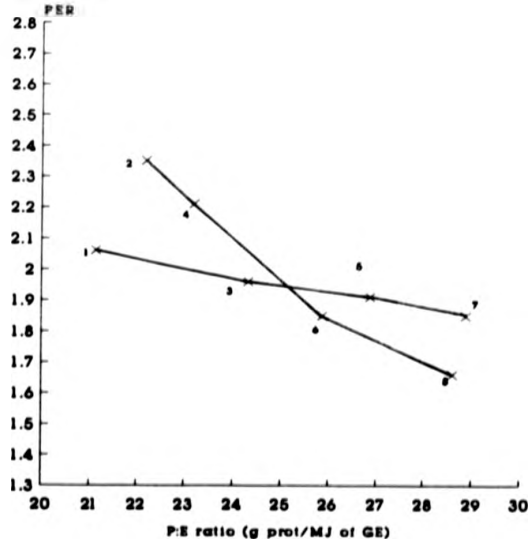
Figure 19. Average final body weight of S.aurata fingerlings against different treatments.

Figure 20.

Specific growth rate (SGR), food conversion efficiency (FCE) and protein efficiency ratio (PER) of S. aurata fingerlings against different treatments.


Specific growth rate (%/day)
3
2.95
2.9
2.85
2.8
2.75
2.7
2.65
2.6
2.55
2.5
2.45
2.4
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)


Food conversion efficiency
1
0.9
0.8
0.7
0.6
0.5
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)


PER
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)

4.3.1.3 Protein Efficiency Ratio

Protein efficiency ratio (PER) was reduced with increasing dietary protein, and for a given dietary protein level, PER showed an increase with increased dietary lipid in diets 1 to 4. On the contrary, in diets 5 to 8 an increase of lipids in the diets produced a decrease in PER values. However, all values were not significantly different ($p < 0.05$) (Table 18, Figure 20).

4.3.1.4 Apparent Net Nitrogen Utilization

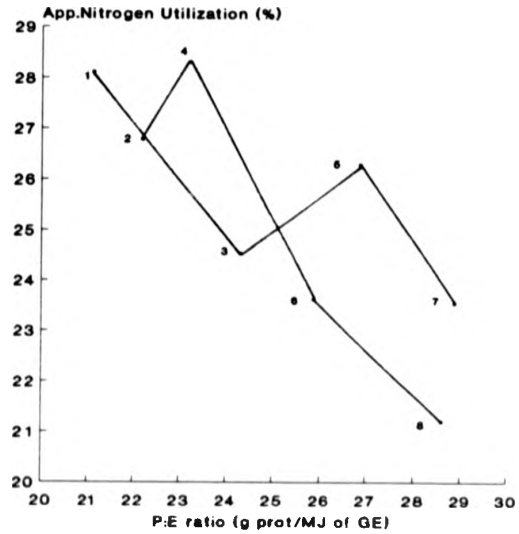
The values of apparent net nitrogen utilization (APNU) obtained show that, as well as PER, there is a reduction in APNU with the increment of protein in the diets. In this case, and for a given dietary protein level, APNU significantly ($p < 0.05$) decreased with increased dietary lipids, except for diets 3 and 4 (47% protein), where an increase of lipids in the diets produced a significant ($p < 0.05$) increment in APNU, this value in diet 4 being the greatest of all diets (Table 18, Figure 21).

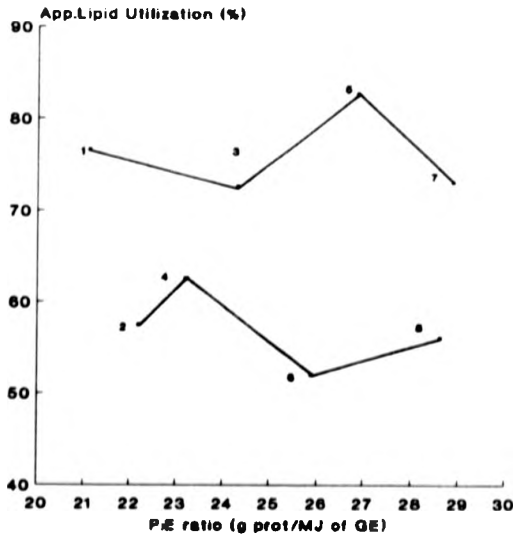
4.3.1.5 Apparent Net Lipid Utilization

The values of apparent net lipid utilization (APLU) obtained showed that there was no apparent trend in APLU values in relation to dietary protein

Figure 21.

Apparent net nitrogen utilization (APNU) and apparent net lipid utilization (APLU) of S. aurata fingerlings against different treatments.


App.Nitrogen Utilization (%)
29
28
27
26
25
24
23
22
21
20
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)


App.Lipid Utilization (%)
90
80
70
60
50
40
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)

content, but for a given dietary protein level, there was a strong reduction in APLU with the increment of lipids in the diets, these values for each protein level being significantly different ($p < 0.05$) (Table 18, Figure 21).

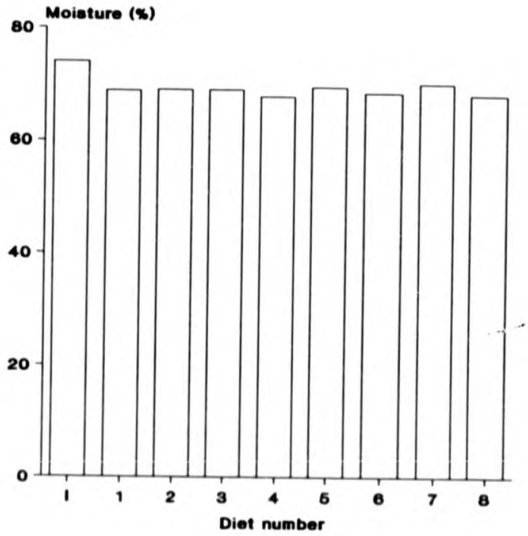
4.3.1.6 Carcass Composition

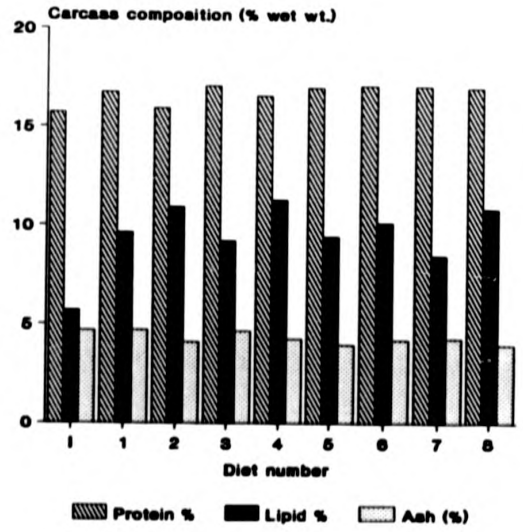
The proximate composition of the whole fish carcass at the start and at the end of the experiment is shown in Table 18. In general, the fish fed the lowest lipid levels for a given protein content tended to have higher moisture, protein and ash contents, but significantly ($p < 0.05$) lower lipid contents (Table 18, Figure 22).

When lipid contents of eviscerated and non-eviscerated fish were compared within each diet, whole fish carcass always had a higher lipid content than that of eviscerated fish carcass, suggesting that *S.aurata* fingerlings normally store excess or reserve fat in the visceral cavity. When these values were compared within each protein level, both eviscerated and non-eviscerated carcass showed an increment in lipid content as dietary lipid increased for all protein levels assayed, suggesting that increased carcass lipid deposition was taking place as dietary lipid increased, and that fat is also stored in non-visceral tissues.

Figure 22.

Carcass moisture, protein, lipid and ash composition (wet weight basis) of S. aurata fingerlings at the start and at the end of the experiment under different treatments.


Moisture (%)
80
60
40
20
0
I 1 2 3 4 5 6 7 8
Diet number


Carcass composition (% wet wt.)
20
15
10
5
0
I 1 2 3 4 5 6 7 8
Diet number
Protein %
Lipid %
Ash (%)

This increase in carcass lipid deposition was higher in viscera than in non-visceral tissues for all protein levels, except in diets containing 52% protein (Table 19).

The proximate composition of fish livers at the end of the experiment is shown in Table 20. In general, fish fed the higher lipid diets showed an increased moisture content, and a decrease in liver protein and lipid. Livers were bigger in fish fed the low lipid diets, except in treatments with 59% protein (Table 20, Figure 23).

When HSI values were related to dietary nutrient levels, a highly positive correlation (Coeff.: 0.83) was found between HSI and carbohydrate levels in diets (the higher dietary carbohydrate, the higher HSI values) ($P < 0.05$).

4.3.1.7 Mortality

Mortality varied from 1.67 to 11.67%. These values can be considered as normal for this species and fish size (Table 18).

Table 19
Carcass lipid content (wet wt.basis) in eviscerated and non-eviscerated fish fed the experimental diets.

DIET NUMBER	1	2	3	4	5	6	7	8
Dietary protein	40.84	44.46	47.86	46.50	53.09	51.81	59.01	58.17
Dietary lipid	8.86	14.87	8.83	14.84	8.81	14.87	8.90	14.89
Carcass lipid content (eviscerated)	8.76[a]	9.49[a]	8.54[a]	10.36[a]	8.33[a]	9.80[a]	7.87[a]	9.98[a]
Carcass lipid content (non-eviscerated)	9.64[b]	10.93[b]	9.21[a]	11.30[b]	9.42[b]	10.14[a]	8.46[a]	10.85[a]
Difference	0.88	1.44	0.67	0.94	1.08	0.35	0.59	0.86

Values in the same column with same superscript are not significantly different ($p < 0.05$)

Table 20
Composition of fish livers at the end of the experiment (wet wt.basis)

DIET	1	2	3	4	5	6	7	8
MOISTURE	62.92	65.29	62.34	66.58	66.41	64.46	64.67	65.13
CRUDE PROTEIN	16.48	15.64	15.67	14.09	13.89	13.07	13.75	12.07
LIPID	8.60	6.31	11.18	6.08	7.13	7.58	8.03	6.21
HSI (%)(1)	1.91[b]	1.55[ab]	1.98[b]	1.57[ab]	1.79[ab]	1.55[ab]	1.38[a]	1.39[a]

(1)HSI = Hepatosomatic index, calculated as (wet liver wt./wet total body wt.) x 100
Note. Values in the same row with same superscripts ae not significantly different ($p < 0.05$)

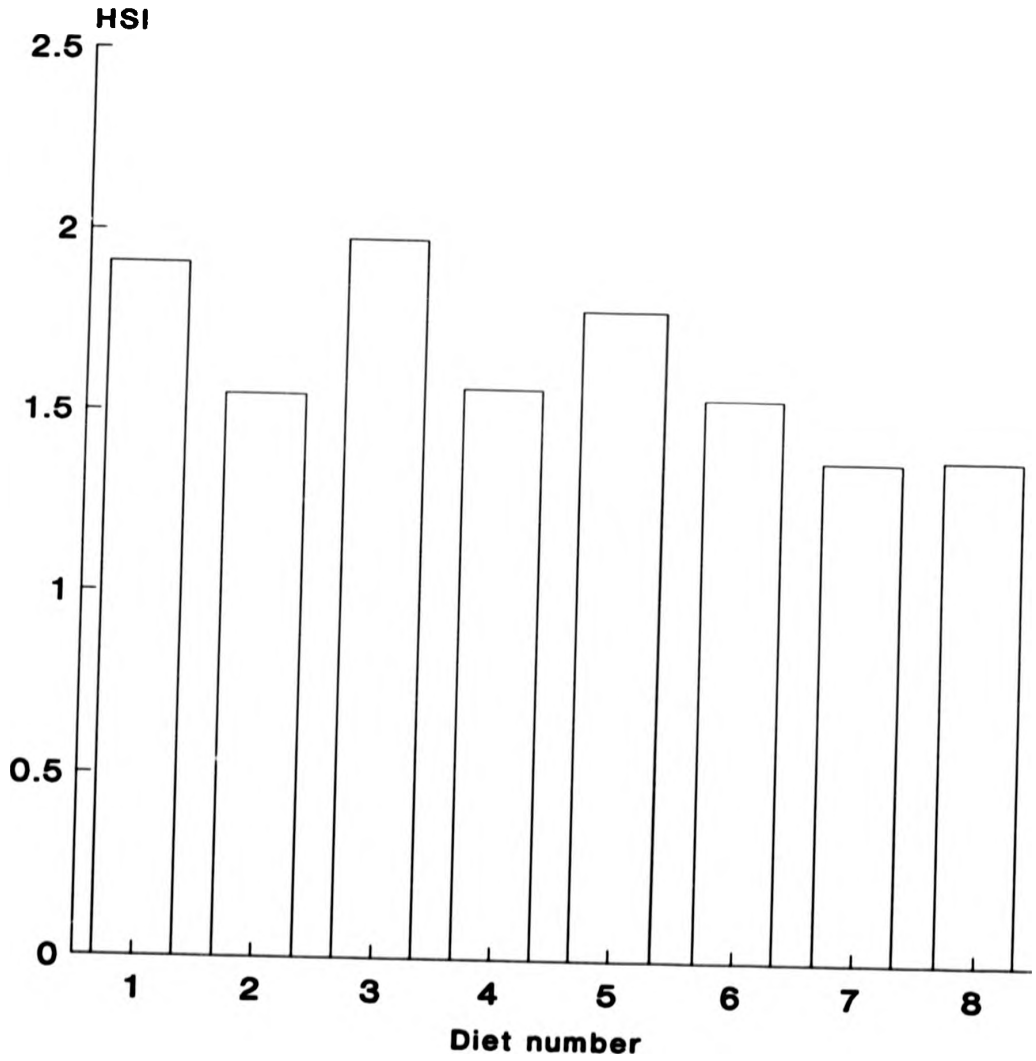


Figure 23. Hepatosomatic indices (HSI) of S.aurata fingerlings at the end of different treatments.

4.3.2 EXPERIMENT IV (Growers)

4.3.2.1 Growth

The growth response of *S. aurata* growers over the experimental period under different protein to energy ratios is shown in Figure 24.

The best growth response in terms of final body weight was obtained with diet 4 (54.2/11.3 = %prot/%lip), with the other diets following in the order: 3 > 2 > 1. No significant difference ($p < 0.05$) was found between diets 2 and 3 (Table 21).

These results suggest that, for a given protein level, fish grew significantly better when dietary lipid level was increased from 7% to 12%. The increase of dietary protein level from 45% to 53% also produced an increment in the growth response (Figure 25).

A similar response was also observed on the basis of percentage weight gain and specific growth rate, the maximum value always being found for diet 4 (Table 21, Figure 26).

4.3.2.2 Food Conversion Efficiency

As both dietary protein and lipid levels increased, food conversion efficiency increased. The value for diet 1 (46.1/7.9 = %prot/%lip) was the lowest

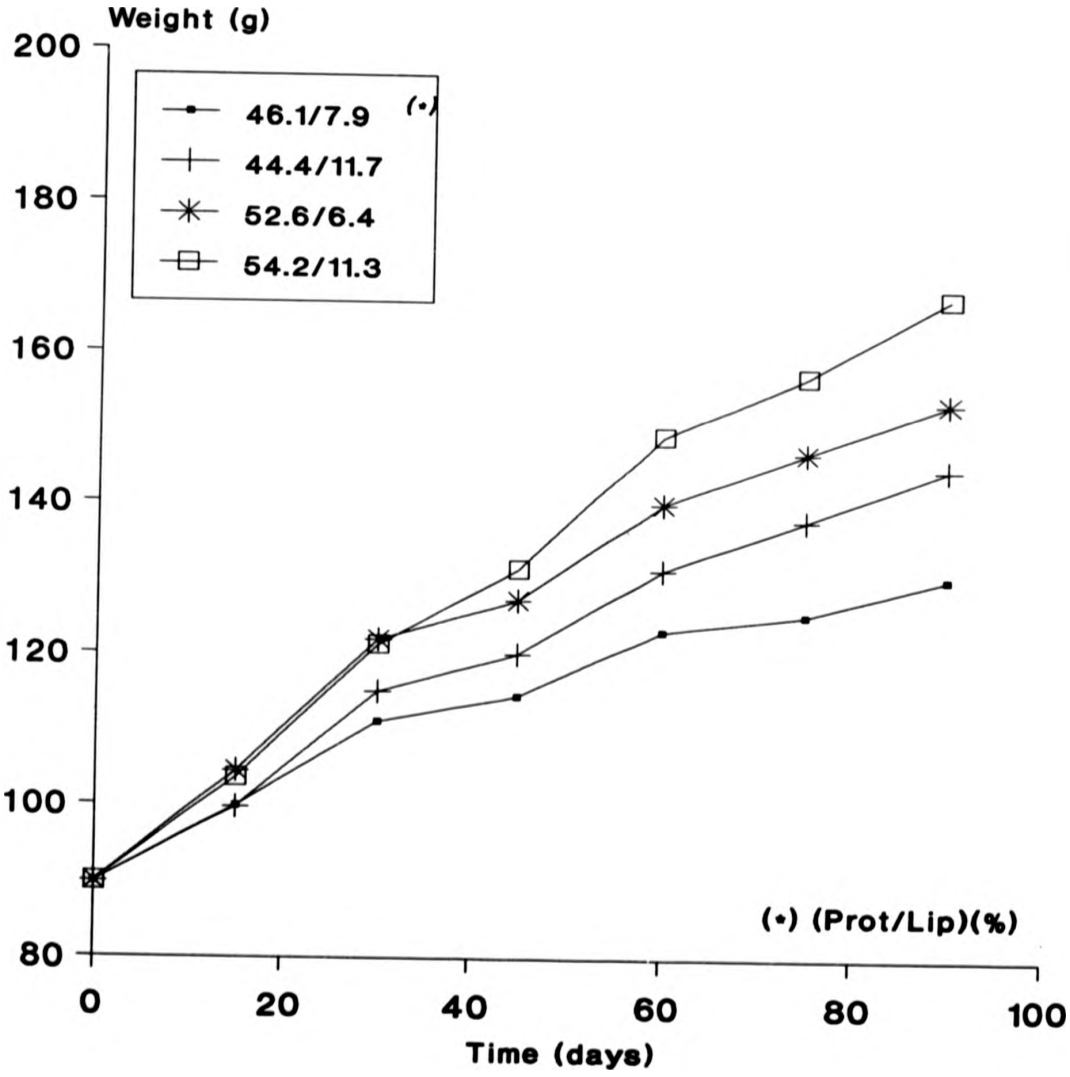


Figure 24. Growth response of S.aurata growers over the experimental period under different protein and lipid levels

Table 21
Mean growth performance, feed utilization efficiency and carcass composition of S.aurata growers fed the experimental diets.

MEAN VALUE RESULTS (Std. error in parenthesis)		DIET NUMBER		
(Prot/Lip) (%)	1 (46.1/7.9)	2 (44.4/11.7)	3 (52.6/6.4)	4 (54.2/11.3)
INITIAL BODY WT. (g) (2.9)	89.8	89.8	89.8	89.8
FINAL BODY WT. (g)	130.2^{a} (2.19)	144.8^{b} (3.04)	153.9^{b} (2.52)	167.5^{c} (2.36)
WEIGHT GAIN (%)	45.0^{a} (2.44)	61.2^{b} (3.39)	71.4^{b} (2.81)	86.6^{c} (3.47)
SGR (%/day) (1)	0.43^{a} (0.02)	0.56^{b} (0.03)	0.64^{b} (0.02)	0.74^{c} (0.02)
FOOD FED (g/day)*	1.57^{a} (0.03)	1.66^{ab} (0.04)	1.78^{b} (0.04)	1.77^{b} (0.05)
WEIGHT GAIN (g/day)*	0.45^{a} (0.05)	0.56^{ab} (0.08)	0.87^{b} (0.17)	0.89^{b} (0.07)
FCE (2)*	0.29^{a} (0.03)	0.34^{ab} (0.04)	0.49^{b} (0.06)	0.50^{b} (0.05)
PER (3)	0.63^{a} (0.08)	0.77^{a} (0.10)	0.94^{a} (0.18)	0.96^{a} (0.09)
APNU (%)(4)*	13.90^{a}	18.57^{b}	18.03^{b}	21.21^{c}
APLU (%)(5)*	41.23^{a}	41.98^{a}	64.44^{c}	53.63^{b}
CARCASS COMPOSITION (% WET WT.BASIS)				
	INITIAL			
MOISTURE*	66.71	67.18^{c}	66.24^{bc}	67.15^{c}	65.62^{b}
CRUDE PROTEIN*	17.09	17.66^{a}	17.80^{ab}	18.29^{ab}	18.50^{b}
LIPID*	10.05	10.22^{ab}	10.99^{b}	9.84^{a}	10.76^{ab}
ASH	6.15	4.94	4.97	4.72	5.12
MORTALITY (%)		3.33	1.66	1.11	2.22

(1)SGR = Specific growth rate, (2)FCE = Food conversion efficiency, (3)PER = Protein efficiency ratio,(4)APNU = Apparent nitrogen utilization, (5)APLU = Apparent lipid utilization.
* Kruskal-Wallis analysis ($p < 0.05$)
Note. Values in the same row with same superscripts are not significantly different ($p < 0.05$).

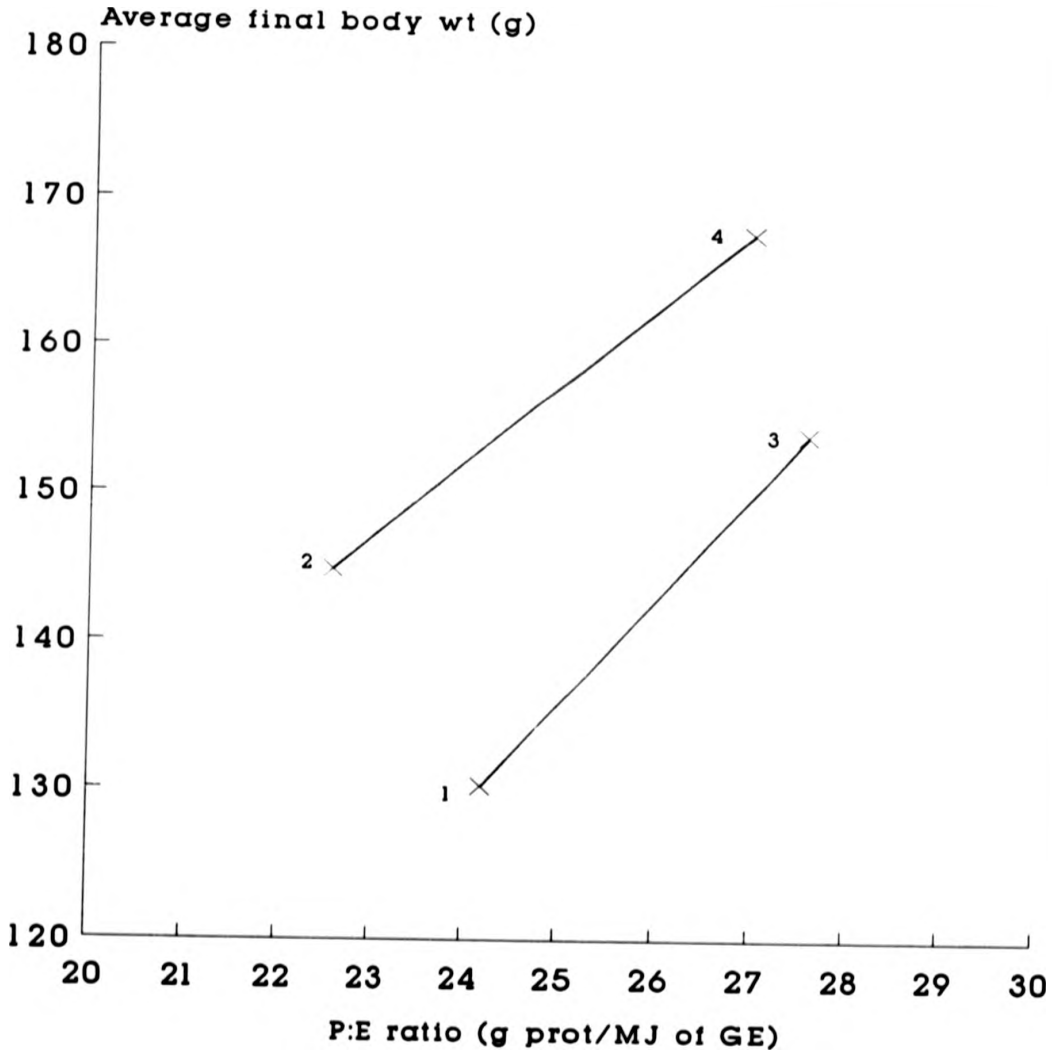
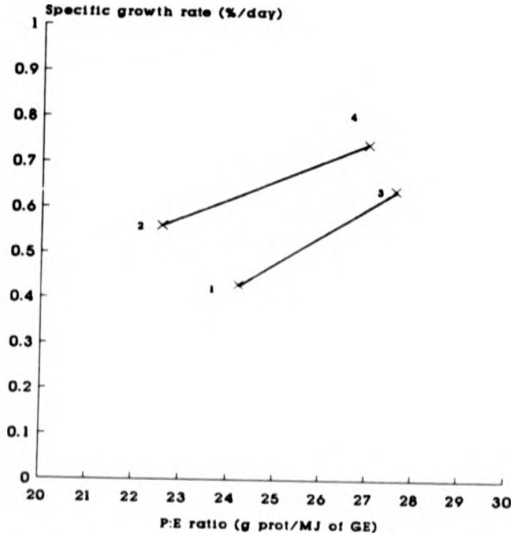
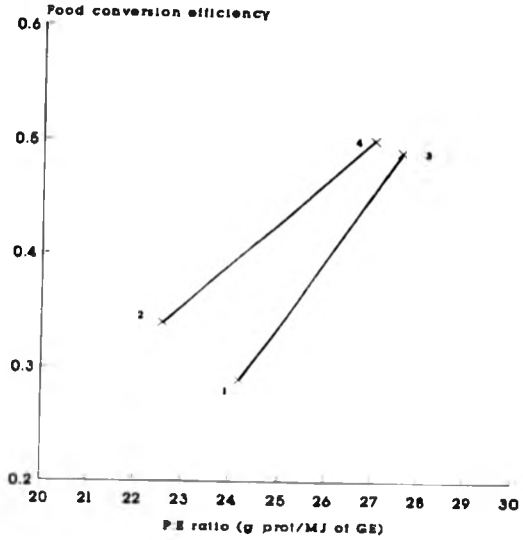


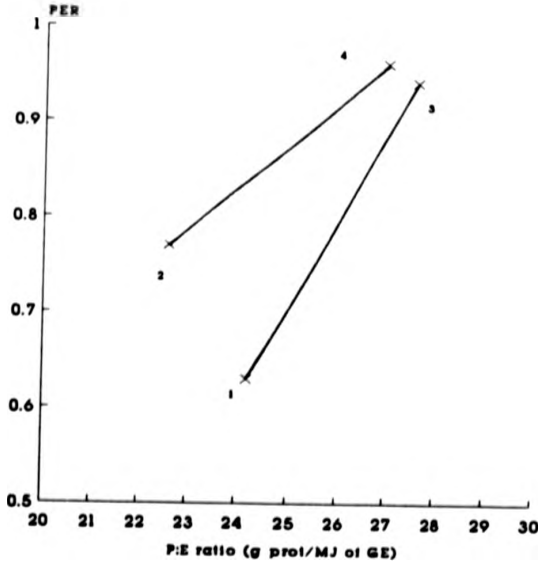
Figure 25. Average final body weight of S.aurata growers against different treatments.

Figure 26.

Specific growth rate (SGR), food conversion efficiency (FCE) and protein efficiency ratio (PER) of S. aurata growers under different treatments.


Specific growth rate (%/day)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)
4
3
2
1


Food conversion efficiency
0.6
0.5
0.4
0.3
0.2
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)
4
3
2
1


PER
1
0.9
0.8
0.7
0.6
0.5
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)
4
3
2
1

and significantly ($p < 0.05$) different from the other diets, except for diet 2 (44.4/11.7 = %prot/%lip) (Table 21, Figure 26).

4.3.2.3 Protein Efficiency Ratio

Protein efficiency ratio (PER) showed an increment with increasing dietary protein, and for a given dietary protein level, PER also increased with increased dietary lipid. However, all values were not significantly different ($p < 0.05$) (Table 21, Figure 26).

4.3.2.4 Apparent Net Nitrogen Utilization

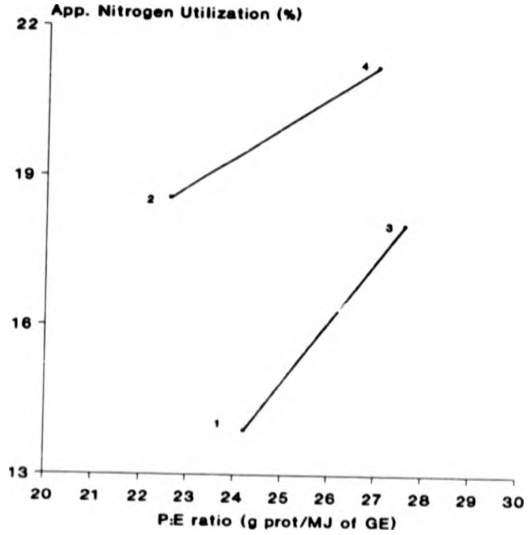
Apparent net nitrogen utilization (APNU) values showed, as well as PER, an increase with both increased dietary protein and lipid. In this case, APNU values for diets 2 and 3 were not significantly different ($p < 0.05$) (Table 21, Figure 27).

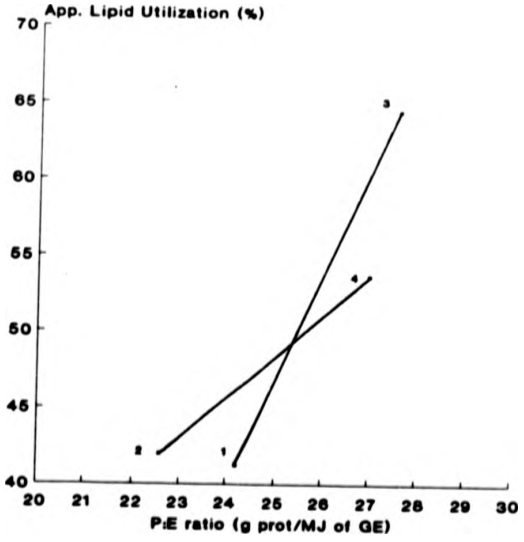
4.3.2.5 Apparent Net Lipid Utilization

Apparent net lipid utilization (APLU) showed an increment with increased protein in the diets. When dietary protein was 45%, an increase in dietary lipid from 7% to 12% supported a slightly increased APLU, but with diets containing 53% protein, the same lipid increment lead to a significantly lower ($p < 0.05$) APLU (Table 21, Figure 27).

Figure 27.

Apparent net nitrogen utilization (APNU) and apparent net lipid utilization (APLU) of S. aurata growers under different treatments.


App. Nitrogen Utilization (%)
22
19
16
13
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)
1
2
3
4


App. Lipid Utilization (%)
70
65
60
55
50
45
40
20 21 22 23 24 25 26 27 28 29 30
P:E ratio (g prot/MJ of GE)
1
2
3
4

4.3.2.6 Carcass Composition

The proximate composition of the whole fish carcass at the start and at the end of the experiment is shown in Table 21.

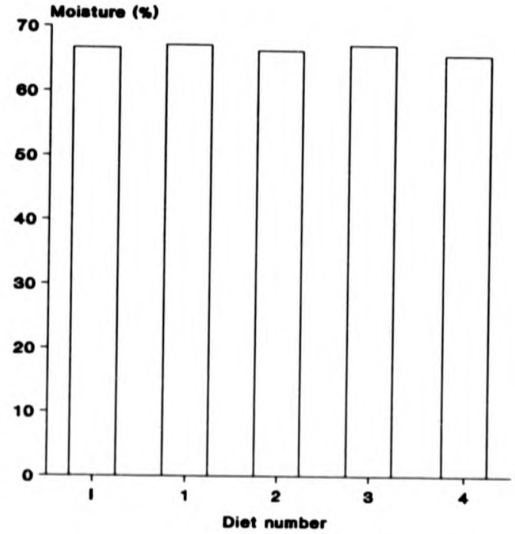
In general, the fish fed the lowest lipid levels for a given protein level tended to have significantly ($p < 0.05$) higher moisture contents, and significantly lower protein and lipid contents. Ash content decreased in fish fed the low lipid diets (Table 21, Figure 28).

When lipid contents of eviscerated and non-eviscerated fish were compared within each diet, whole fish carcass had a higher lipid content than that of eviscerated fish carcass, except in diet 4, suggesting that *S.aurata* growers, as gilthead sea bream fingerlings, normally store excess or reserve fat in visceral tissue. When these values were compared within each protein level, both eviscerated and non-eviscerated carcass showed an increment in lipid content as dietary lipid increased for the two protein levels assayed, suggesting that increased lipid carcass deposition was taken place as dietary lipid increased, and that fat is also stored in non-visceral tissues.

This increase in lipid carcass deposition was higher in viscera than in non-visceral tissues for all protein levels, except in diet 4, when compared with diet 3 (Table 22).

Figure 28.

Carcass moisture, protein, lipid and ash composition (wet weight basis) of S. aurata growers at the start and at the end of the experiment under different treatments.


Moisture (%)
70
60
50
40
30
20
10
0
I
1
2
3
4
Diet number

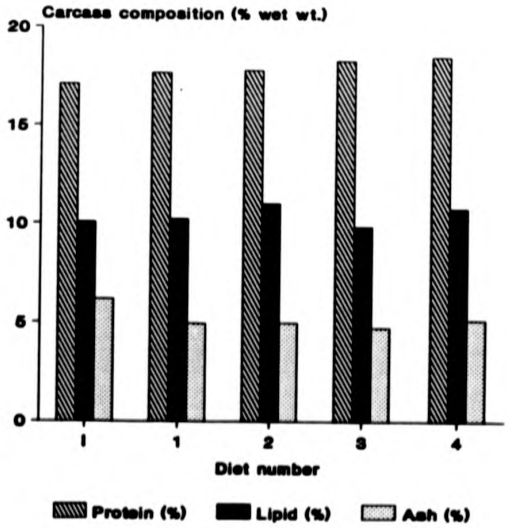

Carcass composition (% wet wt.)
20
15
10
5
0
I
1
2
3
4
Diet number
Protein (%)
Lipid (%)
Ash (%)

Table 22
Carcass lipid content (wet wt.basis) in eviscerated and non-eviscerated fish fed the experimental diets.

DIET NUMBER	1	2	3	4
Dietary protein	46.13	44.38	52.58	54.22
Dietary lipid	7.87	11.75	6.36	11.29
Carcass lipid content (eviscerated)	10.19[a]	10.43[a]	9.14[a]	10.99[a]
Carcass lipid content (non-eviscerated)	10.22[a]	10.99[a]	9.84[a]	10.80[a]
Difference	0.04	0.56	0.70	0.19

Values in the same column with same superscript are not significantly different ($p < 0.05$)

Table 23
Composition of fish liver at the end of the experiment (wet wt.basis)

DIET	1	2	3	4
MOISTURE	68.09	60.60	63.56	64.30
CRUDE PROTEIN	12.48	11.32	11.24	12.70
LIPID	8.44	15.44	11.62	8.05
HSI (%)(1)	1.45[ab]	1.57[ab]	1.75[bc]	1.34[a]

(1)HSI = Hepatosomatic index, calculated as (wet liver wt./wet total body wt.) x 100
Note. Values in the same row with same superscript are not significantly different ($p < 0.05$)

The proximate composition of fish livers at the end of the experiment is shown in Table 23. In general, liver moisture and protein contents were not greatly affected by different treatments. Liver lipid content was lower for fish fed the higher lipid diets with 53% protein (diets 3 and 4), but increased as dietary lipid when the dietary protein level was 45%. Liver size increased in treatment with 45% protein as dietary lipid increased, but decreased significantly ($p < 0.05$) with 53% protein when dietary lipid was incremented from 6% to 11% (Figure 29, Table 23).

No correlation was found between HSI and dietary nutrient levels.

4.3.2.7 Mortality

Mortality varied from 1.11% to 3.33%. These values can be considered as normal for this species and fish size (Table 21).

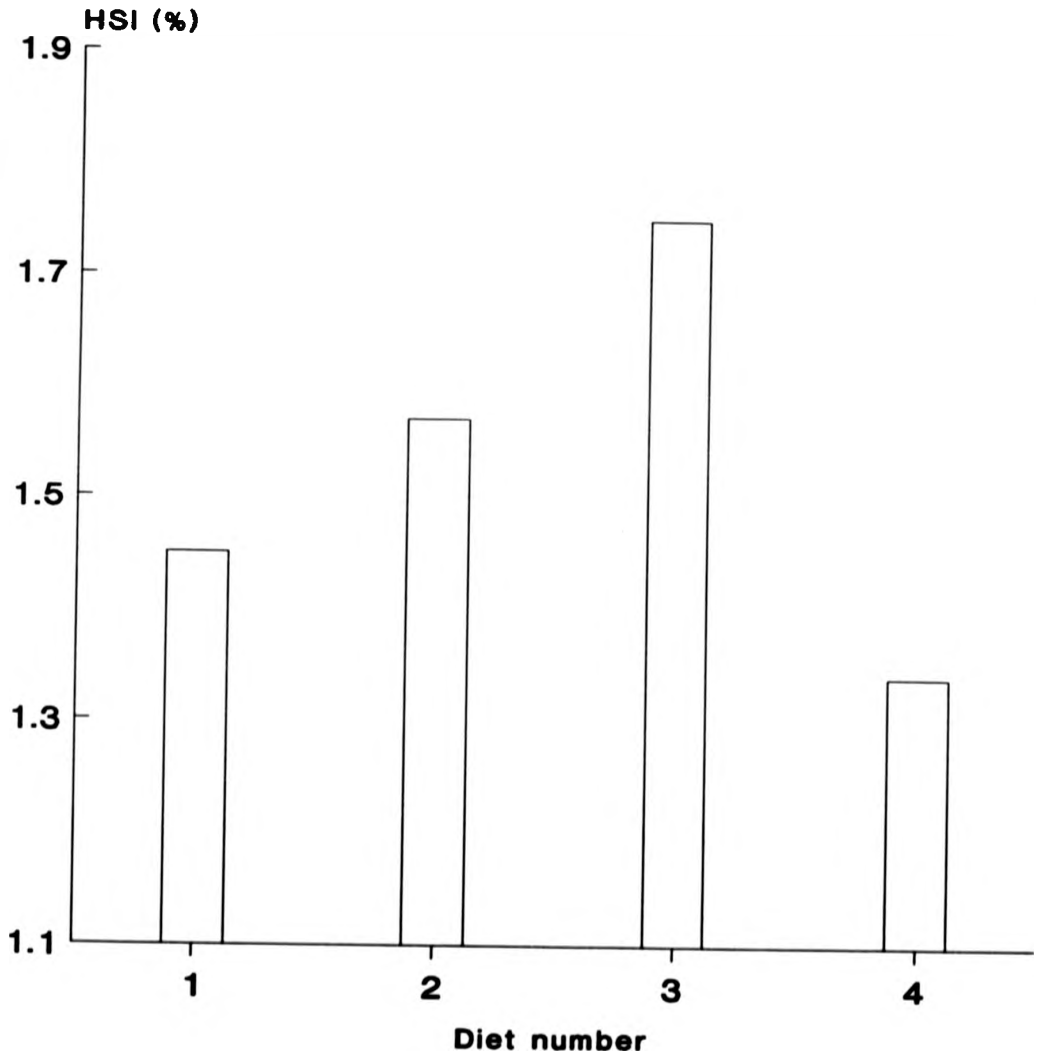


Figure 29. Hepatosomatic indices (HSI) of S.aurata growers at the end of the experiment under different treatments.

4.4 DISCUSSION

In general, growth response of *S. aurata* fingerlings and growers was improved as dietary protein increased up to 52% and 53%, respectively. Furthermore, the increment of dietary lipid within each protein level supported better growth in both fish sizes.

In the second experiment, the growth response of fish to the experimental diets appears poor when compared to that of smaller fish sizes. However, Fernandez-Palacios *et al.*(1989) report that *S.aurata* growers of similar initial body weights (97g) showed an average weight gain value (g/day) of 0.77 (Range: 0.59-1.02) when fed different commercial diets in the Canary Islands. In the present work, the average weight gain (g/day) was 0.69 (Range: 0.45-0.89).

In the experiment with fingerlings, weight gain (g/day) improved as dietary lipid increased up to 52% dietary protein level, and food conversion efficiency (FCE) was similarly better as dietary lipid increased up to 46% protein in the diets. In growers, both weight gain and FCE improved as dietary lipid increased up to 53% dietary protein.

In contrast, Marais and Kissil (1979) found that diets containing 43/9 (%prot/%lip) supported better growth than diets with 43/16 (%prot/%lip) in 44g *S.aurata* juveniles. However, the use of soy bean oil as the main source of non-protein energy in these experiments could have depressed growth and feed

efficiency, as soy bean oil has been reported to reduce voluntary feeding level in *S.aurata* (Kissil and Gropp, 1984), and in general, is considered as a poor source of highly unsaturated fatty acids (HUFA) which are essential in the diet of marine carnivorous fish species (Kanazawa, 1985; Watanabe, 1987; Tacon, 1990).

In studies by Marais and Kissil, weight gain (g/day) decreased from 0.5 to 0.39 and FCE from 0.48 to 0.45 when dietary lipid levels increased from 9 to 16%. In addition, these authors used a fixed dietary protein level in all treatments (43%), giving little room for comparisons with results in the present work in terms of dietary protein levels.

Kissil and Gropp (1984) reported best growth in 3g *S. aurata* fingerlings with diets containing 44/10 (%prot/%lip) and in 45g juveniles with diets containing 40/5 (%prot/%lip), and, despite the fact that they used fish oil as the main source of non-protein energy, the optimum protein levels found in both fish sizes correspond to the maximum levels of the experimental ranges employed (36-40% protein in juveniles, and 40-44% in fingerlings).

Most recently, Pereira *et al.* (1987) found that by increasing dietary protein level from 46% to 51% the growth of 1.5g *S. aurata* fingerlings improved with diets containing a fixed 14% lipid level. Growth rate (g/day) increased from 4 to 14%, and FCE from 0.16 to 0.67. Again, the optimum protein level (51%) was the maximum of the range employed, and the fixed lipid level makes

comparisons difficult. However, fish meal was the only source of dietary protein, in contrast with the previous two mentioned works which used a mixture of fish meal, meat meal and soy bean meal (Marais and Kissil, 1979), and fish meal, meat and poultry meal, soy bean meal, feather meal, fish concentrate protein and soy bean protein (Kissil and Gropp, 1984).

Working with a similar species, the red sea bream (*Chrysophrys major*), Takeuchi *et al.*(1991) fed 2-7g fingerlings diets with a range of 38 to 53% protein and 5 to 20% dietary lipid. They found that the optimum growth performance was obtained with a combination of 52% protein and 15% lipid, when white fish meal plus casein and pollack liver oil were used as sources of dietary protein and lipid, respectively. In that work, weight gain (g/day) improved from 0.25 to 0.37, and FCE from 0.55 to 0.77, when dietary protein level was increased from 38 to 53% in a diet containing 15% lipid. When dietary lipid increased from 5 to 20% in a diet containing a fixed 52% protein level, weight gain (g/day) improved from 0.33 to 0.51, and FCE from 0.73 to 1.03.

When comparing these results with other carnivorous fish species, an increase of dietary lipids usually results in an improved growth and food conversion efficiency, as with *Dicentrarchus labrax* (Alliot *et al.*, 1979, Metailler *et al.*, 1981) and *Anguilla anguilla* (Degani, 1986), best results being frequently obtained with dietary lipid levels of 15%-18%, as reported for *Seriola quinqueradiata* (Shimeno *et al.*, 1980), *Morone saxatilis* (Berger and Halver, 1987) and *Oncorhynchus mykiss* (Watanabe *et al.*, 1979, Alsted and Jokumsen, 1989).

In the present work, this improvement in growth suggests that some sparing effect of lipids on dietary protein could have taken place, when an increase of dietary lipids from 9% to 15% and a decrease in dietary protein from 52% to 46% supported better growth performance in *S.aurata* fingerlings (diets 4 and 5), showing no significant difference from diet 6 which gave best results (Table 18, Figs.18, 19 and 20).

Similarly, an increase of dietary lipid from 6% to 12% and a decrease in dietary protein from 53% to 44% supported no significantly different growth in growers (diets 2 and 3), although in this case significantly best results were obtained with the experimental diet 4 (54/11 = %pro/%lip) (Table 21, Figs 24, 25 and 26).

The protein efficiency ratio (PER) showed an increment as dietary lipid increased both in fingerlings and growers (Figs.20 and 26). Similarly, apparent net nitrogen utilization (APNU) increased in all treatments as dietary lipid increased in growers (Figure 27), and in diets 3 and 4 in fingerlings (Figure 21). Similar observations were described for *Dicentrarchus labrax* (Metailler *et al.*, 1981), *Seriola quinqueradiata* (Takeda *et al.*, 1975; Shimeno *et al.*, 1980), *Scophthalmus maximus* (Bromley, 1980), *Morone saxatilis* (Millikin, 1982) and *Oncorhynchus mykiss* (Higuera *et al.*, 1977; Takeuchi *et al.*, 1978).

With respect to *S. aurata*, Marais and Kissil (1979) found little or no effect of increased dietary lipids (9 to 16%) on PER value (1.03 to 1.07) and a decrease in APNU (From 37.7 to 36.5) for a fixed 43% dietary protein level.

Pereira *et al.*(1987) reported an increased PER value (From 0.3 to 1.3) when fish were fed diets with increased dietary protein from 45.7 to 51% and a fixed 14% lipid content.

Takeuchi *et al.*(1991) found no effect in PER values for a range of dietary protein contents (38 to 53%) and a fixed 15% dietary lipid, and a slight increase in PER (1.8 to 1.9) when dietary lipids were increased from 10 to 20% in a diet with a fixed 52% protein content.

These effects can be interpreted as a substantially higher protein utilization as an increased amount of dietary protein is directed to growth when dietary lipid levels are increased, leading consequently to higher PER and APNU values.

There was a positive correlation between higher dietary lipids and carcass lipid contents, and a negative correlation with carcass moisture and protein contents in fingerlings (Table 18, Figure 22). *S. aurata* growers showed a similar increment in carcass lipid content and a decrease in moisture, although carcass protein content tended to increase with higher lipid levels in diets (Table 21, Figure 28). Overall, the most significant correlation was found in both fish sizes

with carcass lipid content, and similar results have also been reported for other species such as *Chrysophrys major* (Yone *et al.*, 1971), *Dicentrarchus labrax* (Alliot *et al.*, 1979), *Pleuronectes platessa* (Cowey *et al.*, 1975), *Scophthalmus maximus* (Bromley, 1980), *Seriola quinqueradiata* (Shimeno *et al.*, 1980) and *Oncorhynchus mykiss* (Reinitz *et al.*, 1978; Steffens, 1987). Marais and Kissil (1979) also reported an increase in carcass lipid contents (From 11.9 to 12.5%) in *S. aurata* fed a range of dietary lipid levels of 8 to 16%.

In red sea bream, carcass lipid levels rose from 10.6 to 14.5% when fed a range of dietary lipid levels from 10 to 20% (Takeuchi *et al.*, 1991).

In the present work, the average increment in carcass lipid content was from 9.2 to 10.8% for a dietary lipid increase of 9 to 15% in fingerlings, and from 10.0 to 10.8% for a dietary lipid increase of 7 to 11% in growers.

This effect is widely interpreted as an increase in carcass lipid deposition, which may not be always desirable (Cowey and Sargent, 1979).

When lipid contents of eviscerated and non-eviscerated fish were compared, the results suggest that fat was increasingly incorporated both in viscera and in non-visceral tissues as dietary lipid increased, but at a comparatively higher rate in non-visceral tissues with 52% and 53% protein in fingerlings and growers, respectively. This may indicate an undesirable increment of fat in fish muscle tissues for these treatments. However, results

reported for wild *S.aurata* individuals from the Mediterranean coasts of average weight 150g indicate that average lipid contents for eviscerated and non-eviscerated wild fish are 12.9% and 13.6%, respectively (Echevarria *et al.*, 1987). These values are higher than those obtained in the present experiments (Tables 19 and 22).

Apparent net lipid utilization (APLU) values showed a decrease as dietary lipid was increased in both experiments (Tables 18 and 21, Figures 21 and 27). This may indicate that a higher percentage of dietary lipids was used as fuel hence improving the protein utilization by fish.

Liver lipid content decreased as higher levels of lipids were used in both experiments, except in growers with diets 1 and 2. This could be interpreted as an adaptation of fish intermediary metabolism to dietary composition. In this case, a reduction in the net synthesis of fat, by decreasing the liver fatty acid synthesis from non-fatty precursors, as sufficient ready-made fat for intermediary metabolic processes was already present, could explain the above values. Consequently, the energy which was otherwise required for "de novo" synthesis of fatty acids from acetyl-CoA was freed for other purposses and this enhanced the degree of protein utilization (Steffens, 1989).

Thus, when rainbow trout was fed diets containing two dietary lipid levels (8 and 17%) and a fixed 46% protein content, the activity of glucose-6-

phosphate dehydrogenase, a liver enzyme involved in fatty acid synthesis, decreased from 306 mU/mg to 167 mu/mg (Sanchez-Muros, 1990).

Liver size was positively correlated with carbohydrate levels in fingerlings, and in both experiments decreased as dietary lipid increased (Tables 19,20,22 and 23).

Mortality of fish during both experiments cannot be associated with different treatments.

Overall, these findings suggest that best protein to energy ratio (P:E) for *S. aurata* fingerlings was 21.9 g protein/MJ of gross energy for a diet containing 46.5% protein and 14.8% lipid, and that some sparing effect of dietary lipid on protein was taking place. Best P:E ratio for *S. aurata* growers was 26.38 g protein/MJ of gross energy for a diet containing 54% protein and 11% lipid, with less apparent protein sparing effect of dietary lipids.

Lipid carcass deposition was produced in both fish sizes but it was not excessive when compared to wild animals.

Differences with previous results reported for gilthead sea bream are explained based on the use of vegetable oils as dietary lipid sources by some authors (Marais and Kissil, 1979), which may have produced diets with deficiency in essential fatty acids (EFA), hence depressing growth and feed

efficiency. In addition, the use of restrictive variable ranges and different feed ingredients (Marais and Kissil, 1979; Kissil and Gropp, 1984) may also have influenced different results obtained. When similar feed ingredients to those employed in this work were used with *S.aurata* and *C.major*, results seem to coincide in the suggestion that *S.aurata* appears to improve its performance under high energy and high protein diets (Pereira *et al.*, 1987; Takeuchi *et al.*, 1991).

Similar findings have been reported for *Seriola quinqueradiata* (Takeda *et al.*, 1975), *Morone saxatilis* (Millikin, 1983) and *Oncorhynchus mykiss* (Alsted and Jokumsen, 1989).

Results obtained in the present work also support optimum protein requirements found in the previous chapter, although the requirements for protein of growers were slightly higher than expected. Further research work may contribute to confirm the results, perhaps by using a wider range of dietary protein contents, including lower dietary protein levels.

5. EVALUATION OF DIFFERENT FEEDSTUFFS AND DIETARY LIPID LEVELS AND THEIR INFLUENCE ON NUTRIENT DIGESTIBILITY AND DIETARY ENERGY UTILIZATION IN *S.aurata* JUVENILES

5.1 INTRODUCTION*

One of the most important aspects in the evaluation of the biological effectiveness of a feedstuff is the determination of its digestibility (Phillips, 1969; Cho, 1987). This measures the ability of the fish to digest and absorb the nutrients it is fed.

It has been shown that considerable variation exists in the nutrient digestibility of different feedstuffs between fish species (Lovell, 1977; Stickney, 1979). Fish are well able to digest fats and proteins (Takeuchi, 1979; Law *et al.*, 1983), but are poorly adapted to digest carbohydrates (Cowey and Sargent, 1979; Shimeno, 1982; Spanhof and Plantikow, 1983; Anderson *et al.*, 1984).

Other carbohydrates such as fibres, hemicellulose, lignin and pentosans generally form undigestible fractions in the feed. The growth of some fish species tends to be depressed by the presence of about 8% of dietary fibre and is highly depressed when the fibre content reaches 20%, probably due to the dilution of digestible nutrients through increased bulk or by obstruction of enzyme action (N.R.C., 1983). Particle size, source and nature of fibre present in the diet are also factors determining its utilization by fish (Davies, 1985).

* This chapter was presented as a poster in the IV INTERNATIONAL SYMPOSIUM ON FISH NUTRITION AND FEEDING, June 24-27, 1991. Biarritz, France.

In addition, there may also be antinutrients found in natural feedstuffs, which can produce problems in the absorption of nutrients through the inhibition of some chemical mechanisms utilized during digestion, as for example, the amylase and protease inhibitors found in raw wheat (Tacon, 1985).

Considerable differences in carbohydrate digestibility between various fish species have been reported, as could be expected as a consequence of the marked variations in the anatomy of the digestive tract and in the native diet (Steffens, 1989).

As stated in the general introduction, carnivorous fish species have a limited capacity to digest carbohydrates, and high contents of dietary carbohydrate have been reported to negatively affect protein digestibility, feed efficiency and growth, increase liver glycogen deposition, hepatosomatic index and even caused eventual mortality (Shimeno *et al.*, 1979; Kaushik *et al.*, 1989).

Relative nutrient proportions in the diet also affect digestibility, and increased amounts of dietary lipids are known to support increased protein, carbohydrate, lipid and energy digestibility (Hepher, 1988)

The aim of the present work was to study the effect of different dietary carbohydrate sources and dietary lipid levels on feed digestibility and dietary energy utilization in *S. aurata* juveniles.

5.2 MATERIALS AND METHODS

For these two trials (V and VI), gilthead sea bream juveniles of mean body weight 41.9g and 46.4g were employed, respectively. Experiments were not repeated with smaller fish because of the foreseen major difficulty in obtaining enough faecal material. Experimental fish were stocked at 10 fish per tank, three tanks per treatment.

Prior to starting the experiments, fish were acclimated for one week, feeding them with diets containing 54.9/14.7 and 53.9/10.7 (%prot/%lip), respectively, at a rate of 3% of body weight per day. In the first trial, the diet used for fish acclimation had a carbohydrate source similar to those of experimental diets used in Sections 3 and 4. In the second trial, acclimation was carried out with the diet containing a lipid level similar to those of diets in Section 3. At the start of each experiment 10 fish were killed by an overdose of benzocaine and stored at -20°C for chemical analysis.

Tank system III (Section 2.2.3) was utilized in both trials. Table 24 shows the mean values obtained for the environmental parameters recorded during the two experiments, as detailed in Section 2.3. All values were within the optimum ranges for this species (Section 1.6).

In the first trial, four different experimental diets were prepared, containing four different carbohydrate sources: corn starch, dextrin, wheat bran

Table 24
Environmental parameters recorded during digestibility experiments with juveniles.

EXP.V		
Temperature	21.08°C	± 0.06
pH	8.11	± 0.02
Dissolved oxygen	6.81	± 0.32 mg/l
Salinity	36.6	± 0.01 ppt
Nitrite	0.006	± 0.003 mg/l
EXP.VI		
Temperature	19.98°C	± 0.37
pH	8.10	± 0.08
Dissolved oxygen	6.67	± 0.60 mg/l
Salinity	36.6	± 0.02 ppt
Nitrite	0.011	± 0.006 mg/l

and corn starch/dextrin (3/1). All diets had similar protein (53%) and lipid (15%) contents (Table 25).

In the second trial, three different dietary lipid levels (10.7%, 14.1% and 16.3%) were fed to gilthead sea bream juveniles. All diets had a similar protein content (53%). The lipid content was decreased conventionally by replacing sardine oil with dextrin and corn starch in a proportion of 1:3 in order to obtain approximately isoenergetic diets (Table 26).

The diameter of dry pellets was 2mm in both experiments. Tables 25 and 26 show the composition of the experimental diets used in both trials.

The feeding regime used during both experiments was 3% of body weight per day, with a frequency of two times a day, seven days a week. Faeces were not collected until the third day of feeding to be sure that faeces truly corresponded to the diets offered. Care was taken shortly after giving the food to clean the whole system, starting from the bottom of the tanks to the settling columns, to avoid any mixing of faeces and unconsumed food.

Faeces were collected every morning and afternoon before feeding by closing the tank outlet valve and pouring about 250ml of water and faeces from the bottom of the settling columns into glass settling jars. The fish were then fed and the tanks were washed out as described above. After collection, the faeces were placed in aluminium containers and dried overnight at 105°C. The dried

Table 25. Composition of the experimental diets used in the first digestibility experiment (Exp.V) and their respective proximate analysis.

INGREDIENTS (%)	DIET NUMBER 1	2	3	4
Sardine meal (1)	73.2	73.2	73.2	69.5
Corn dextrin	3.5	14.1	-	-
α-Cellulose	-	-	-	3.4
Corn starch	10.6	-	14.1	-
Wheat bran (2)	-	-	-	14.1
Sardine oil	9.3	9.3	9.3	9.5
C.M.Cellulose (3)	0.5	0.5	0.5	0.5
Vitamin premix (4)	2.0	2.0	2.0	2.0
Mineral premix (4)	0.4	0.4	0.4	0.4
Indicator (Cr_2O_3)	0.5	0.5	0.5	0.5
NUTRIENT CONTENT (% DRY WEIGHT)				
Crude protein	54.9	52.9	53.1	52.2
Ether extract	14.7	14.2	15.4	15.5
Ash	14.1	15.1	13.8	14.6
Fibre	0.3	0.3	0.0	4.6
Carbohydrate	15.4	16.9	17.1	12.6
Cr_2O_3	0.5	0.5	0.5	0.5
Gross energy (Mj/Kg)	20.2	19.5	20.1	20.0
Protein:Energy (g : Mj of GE)	27.2	27.1	26.4	26.0

(1) Proximate analysis (% dry wt.): Crude protein: 62.4, Ether extract: 8.3, Fibre: 9.9, Ash: 17.4.
(2) Proximate analysis (% dry wt.): Crude protein: 16.9, Ether extract: 3.9, Fibre: 47.4, Ash: 5.5.
(3) Carboxy methyl cellulose (Sodium salt).
(4) Table 8, Section 2.4.

Table 26. Composition of the experimental diets used in the second digestibility experiment (Exp.VI) and their respective proximate analysis.

INGREDIENTS (%)	DIET NUMBER		
	1	2	3
Sardine meal (1)	73.2	73.2	73.2
Corn dextrin	3.2	1.6	-
α-Cellulose	5.1	8.4	11.7
Corn starch	9.5	4.7	-
Sardine oil	5.7	8.7	11.7
C.M.Cellulose (2)	0.5	0.5	0.5
Vitamin premix (3)	2.0	2.0	2.0
Mineral premix (3)	0.4	0.4	0.4
Indicator (Cr_2O_3)	0.5	0.5	0.5
NUTRIENT CONTENT (% DRY WEIGHT)			
Crude protein	53.9	52.8	53.2
Ether extract	10.7	14.1	16.3
Ash	13.6	13.7	13.7
Fibre	4.9	7.9	11.2
Carbohydrates	16.4	10.9	5.1
Cr_2O_3	0.4	0.4	0.5
Gross Energy (Mj/Kg)	19.7	20.4	21.4
Protein:Energy (g : Mj of GE)	27.4	25.9	24.8

(1) Proximate analysis (% dry wt.): Crude protein: 62.4, Ether extract: 8.3, Fibre: 9.9, Ash: 17.4.
(2) Carboxy methyl cellulose (Sodium salt).
(3) Table 8, Section 2.4.

faeces were placed in a vacuum drier until cool and later powdered in a mortar and pooled with collections corresponding to each diet. This material was then stored at -20°C for chemical analysis.

The fish were maintained under the experimental conditions for 3 weeks in both trials to ensure that enough faecal material was available for all analyses.

At the start and at the end of both experiments fish were individually weighed to the nearest one decimal place (0.1g) on a Mettler top-pan balance (Model PE-3000). At the end of the trials fish were dried in order to obtain total moisture and later the carcasses were finely ground for chemical analysis.

Fish mortality was recorded daily to adjust the amount of food offered per day.

Dietary energy utilization was evaluated using the schedule proposed by Cho and Kaushik (1985). This method involves digestibility analysis and comparative carcass analysis, and is summarized in Table 4, Section 1.5.2.

Partitions of dietary energy were carried out by estimations of digestible energy, recovered energy, metabolizable energy, heat loss and branchial plus urinary energy loss. Energy retention efficiencies were also determined, as shown in Table 4 and Table 30.

5.3 RESULTS

5.3.1 EXPERIMENT V

5.3.1.1 Digestibility

Apparent digestibility coefficients (ADC) of dry matter, nutrients and gross energy for the experimental diets are shown in table 27.

The fact that ash content in faeces were high (Table 27) may indicate that significant amounts of NaCl were present, hence leading to errors when calculating carbohydrate content in faeces. Thus, the latter was re-calculated based upon recorded faeces gross energy content and empirical gross energy content of nutrients (i.e.: The gross energy content of faeces was calculated based on protein, lipid and fibre contents and their typical gross energy values). The difference between this value and the measured faecal gross energy content was used to re-calculate carbohydrate contents). Carbohydrate digestibility coefficients were then also re-calculated accordingly.

Similarly, break-down of faeces during collection may have produced Cr_2O_3 losses, leading to errors in the estimations of dry matter digestibility coefficients (Total digestibility). These values were also re-calculated based on the next equation:

Table 27. Proximate analysis of faeces and apparent digestibility coefficients of fish fed on different diets.

Nutrient content in faeces (% dry matter)	DIET NUMBER 1	2	3	4
Crude protein	10.12	9.19	9.47	8.01
Lipid	0.41	0.05	0.17	0.38
Ash	75.18	75.39	73.98	55.23
Fibre	1.44	1.46	1.23	10.39
Cr_2O_3	1.54	1.35	1.37	1.19
Carbohydrate (*)	11.31 (7.67)	12.56 (11.44)	13.78 (10.06)	24.80 (5.76)
Gross energy ($Mj.Kg^{-1}$)	3.85	7.08	4.02	3.02
APPARENT DIGESTIBILITY COEFFICIENTS (%)				
Dry matter (*)	68.2 (91.4)	65.9 (91.5)	64.9 (90.9)	61.3 (88.3)
Protein	94.1	94.1	93.8	94.1
Lipid	99.1	99.9	99.6	99.0
Carbohydrate (*)	76.7 (84.2)	74.7 (76.9)	71.8 (79.4)	64.2 (82.4)
Gross Energy	93.9	87.6	93.0	94.2

* Re-calculated values in parenthesis, based on measured faeces gross energy content and typical gross energy content of nutrients.

$$(P \times PD) + (L \times LD) + (CHO \times CHOD) + (A \times AD) = (DM \times DMD)$$

Where:
P = Dietary protein
L = Dietary lipid
CHO = Dietary carbohydrate
A = Dietary ash
DM = Dietary dry matter
D = Apparent digestibility coefficient

N.B.- Crude fibre values were not used in re-calculating dry matter digestibility, as fibre digestibility is regarded as nil.

The ADC of dry matter, lipid and crude protein were not greatly influenced by the different ingredients in the diet, but there was a slight decrease in the ADC of dry matter and lipid when a higher level of fibre was present in diet 4. Carbohydrate and gross energy digestibility were lowest in diet 2, where dextrin was used as the only source of carbohydrate. Digestibility of carbohydrate was lower than 85% regardless of carbohydrate source (Table 27, Figure 30).

As fibre content was estimated both in diets and faeces, nutrient digestibility was also calculated based upon this natural internal marker (fibre), in order to compare it with Cr_2O_3 to see what effect had on digestibility values.

Table 28 shows apparent digestibility coefficients (ADC) of dry matter, nutrients and gross energy for the experimental diets when crude fibre was used as inert marker for calculations. The ADC values obtained in this case showed similar trends to those obtained with Cr_2O_3. A slight decrease in the ADC of dry

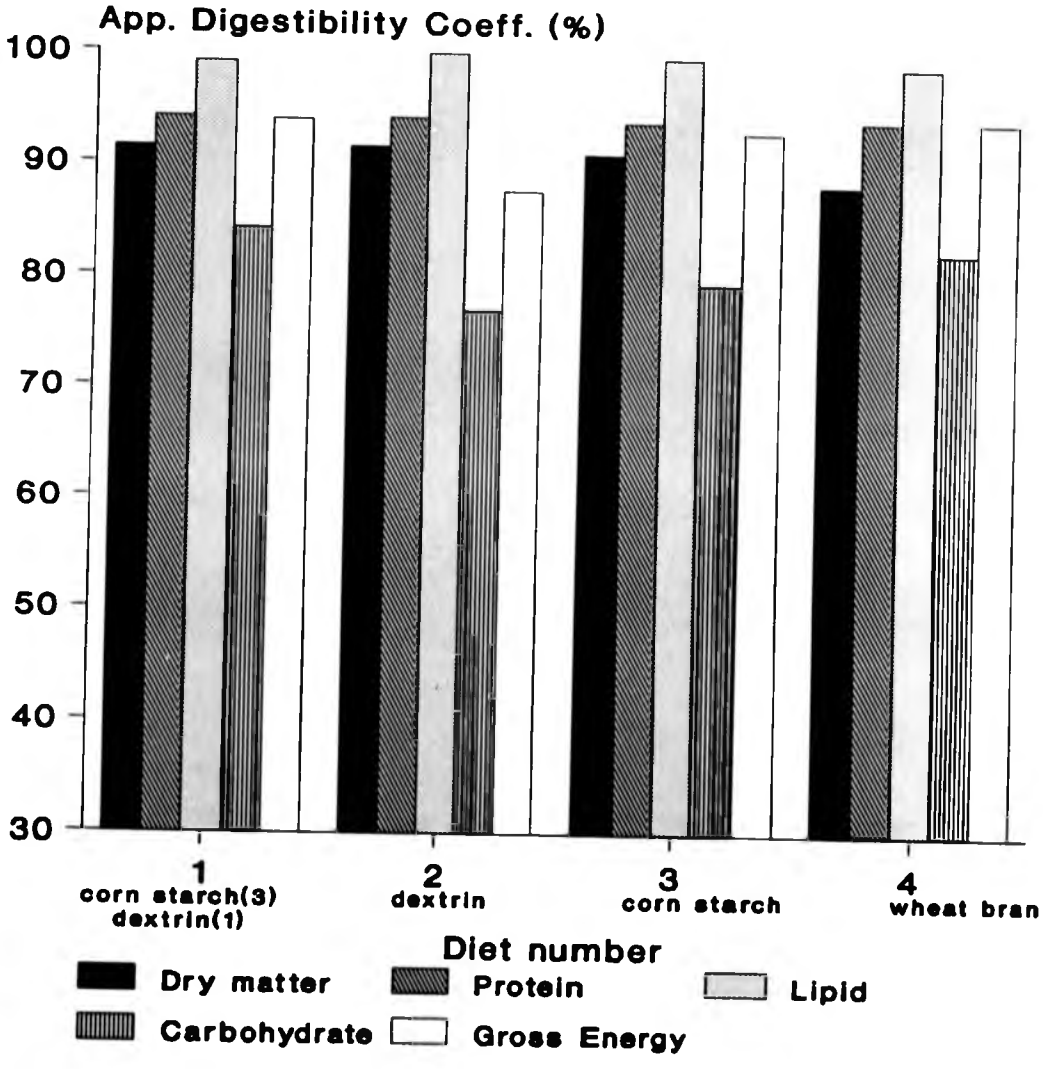


Figure 30. Apparent digestibility coefficients (ADC) of dry matter, protein, lipid, carbohydrate and gross energy under different treatments in the first digestibility experiment.

Table 28. Apparent digestibility coefficients (ADC, %) in experiment V when crude fibre was used as internal marker for calculations.

	DIET NUMBER 1	2	3	4
Dry matter (*)	79.2 (93.4)	79.4 (94.3)	- -	55.7 (87.5)
Protein	96.1	96.4	-	93.2
Lipid	99.4	99.9	-	98.9
Carbohydrate (*)	84.7 (89.6)	84.7 (86.1)	- -	62.8 (79.8)
Gross Energy	96.0	92.5	-	93.3

(*) Re-calculated values in parenthesis (See Table 27).

matter, protein and lipid was apparent when a higher level of fibre was present in diet 4. Carbohydrate digestibility was lowest in diets 4 and 2, and the ADC of gross energy was lowest in diet 2, where dextrin was used as the only source of carbohydrate. The absence of crude fibre in the proximate analysis of diet 3 made it impossible to calculate ADC values for nutrients in this case.

5.3.1.2 Gains

The net gains of live and dry body weights, protein and lipid are shown in Table 29. The effects of different carbohydrate sources on all net gains were not statistically different ($p < 0.05$), although all above values slightly decreased when dextrin was the only source of carbohydrate.

The conversions (intake/gain ratio) of feed and dry matter, protein and lipid of feed are also shown in Table 29. Conversions were not greatly affected by different treatments, but all values were slightly higher in diet 2.

5.3.1.3 Partition of dietary energy and energy retention efficiencies

As shown in Table 30, there were no appreciable differences in non-faecal energy losses (ZE + UE). The diets containing corn starch as the only source of carbohydrate and mixed in a high proportion (3/1) with dextrin produced

Table 29. Effect of different dietary carbohydrate sources on weight gain and conversion in *S.aurata* juveniles (*).

CARBOHYDRATE SOURCE	Corn starch(3) Dextrin (1)	Dextrin (100%)	Corn starch (100%)	Wheat bran (100%)
		(Kg gain/100 fish)		
Live body weight	1.67[a]	1.29[a]	1.66[a]	1.70[a]
Dry body weight	0.65[a]	0.54[a]	0.67[a]	0.59[a]
Protein	0.35[a]	0.30[a]	0.33[a]	0.35[a]
Lipid	0.29[a]	0.24[a]	0.33[a]	0.26[a]
		(intake/gain ratio)		
Feed	1.43[a]	1.80[a]	1.44[a]	1.38[a]
Dry matter	3.68[a]	4.29[a]	3.53[a]	3.92[a]
Protein	3.68[a]	4.07[a]	3.75[a]	3.42[a]
Lipid	1.19[a]	1.35[a]	1.12[a]	1.36[a]

* Data: Initial body weight: 4.199 Kg/100 fish
Note. Values in the same row with same superscript are not significantly different ($p < 0.05$)

Table 30. Partition of dietary energy and energy retention efficiences.

Dietary carbohydrate source	DE	RE	ZE+UE	ME	HE	ERE
			(Mj per Kg feed intake)			
Corn starch Dextrin(3/1)	16.34	11.11	0.03	16.31	5.20	0.68
Dextrin(100%)	12.44	8.20	0.03	12.41	4.21	0.66
Corn starch (100%)	16.09	12.11	0.03	16.06	3.95	0.75
Wheat bran (100%)	17.01	6.98	0.03	16.98	10.00	0.41

DE (Digestible energy) = (GE in food - GE in faeces)

RE (Recovered energy) = (final carcass GE-initial carcass GE)

ZE+UE (Branchial+Urinary energy loss)= 24.9 Mj x (Kg digest. Nintake- Kg recovered N)

ME (Metabolizable energy) = DE - (ZE+UE)

ERE (Energy retention efficiency) = RE/DE.

HE (Total heat loss) = ME - RE

increased ME, RE and energy retention efficiencies (ERE), indicating improved utilization of energy. The diet containing wheat bran as carbohydrate source produced the lowest RE and ERE values, possibly due to the higher amount of fibre.

5.3.2 EXPERIMENT VI

5.3.2.1 Digestibility

Apparent digestibility coefficients (ADC) of dry matter, nutrients and gross energy for the experimental diets are shown in Table 31.

Again, carbohydrate and dry matter digestibility coefficients were re-calculated based on assumptions discussed in experiment V. Proximate analysis of fish faeces is also shown in Table 31.

The ADCs of gross energy, lipid and crude protein increased as dietary lipid increased from 10.75% to 14.15%, but all these ADC values dropped when the lipid level was 16.27%. These effects are interpreted as caused principally by the increasing amount of fibre in the highest lipid diet. Dry matter and carbohydrate digestibilities decreased as dietary lipid increased (Figure 31).

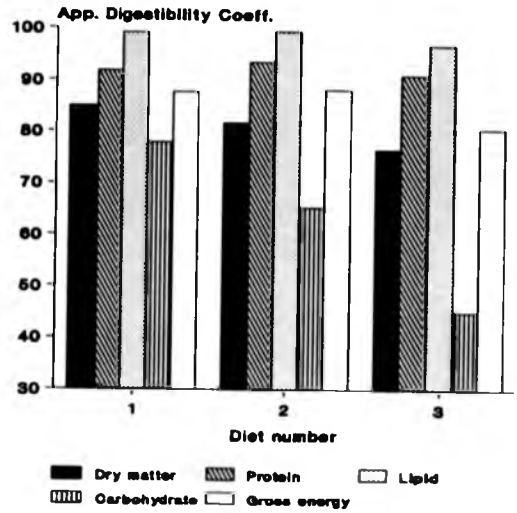
Table 31. Proximate analysis of faeces and apparent digestibility coefficients for fish fed on different diets.

Nutrient content in faeces (% dry matter)	DIET NUMBER 1	2	3
Crude protein	10.29	8.10	7.51
Lipid	0.21	0.18	0.78
Ash	65.57	63.84	57.88
Fibre	9.57	12.55	16.53
Cr_2O_3	1.02	1.02	0.86
Carbohydrate (*)	13.34 (8.43)	14.31 (9.01)	16.44 (9.88)
Gross energy ($Mj.Kg^{-1}$)	5.61	5.70	6.62
APPARENT DIGESTIBILITY COEFFICIENTS (%)			
Dry matter (*)	56.8 (84.9)	57.8 (81.6)	37.2 (76.5)
Protein	91.8	93.5	91.1
Lipid	99.2	99.5	96.9
Carbohydrate (*)	(77.8)	(65.3)	(45.0)
Gross Energy	87.7	88.2	80.6

* Re-calculated values in parenthesis (See Table 27)

Figure 31.

Apparent digestibility coefficients (ADC) and live and dry body weights, protein and lipid gains (Kg/100 fish) under different treatments in the second digestibility experiment.


App. Digestibility Coeff.
100
90
80
70
60
50
40
30
1
2
3
Diet number
Dry matter
Protein
Lipid
Carbohydrate
Gross energy

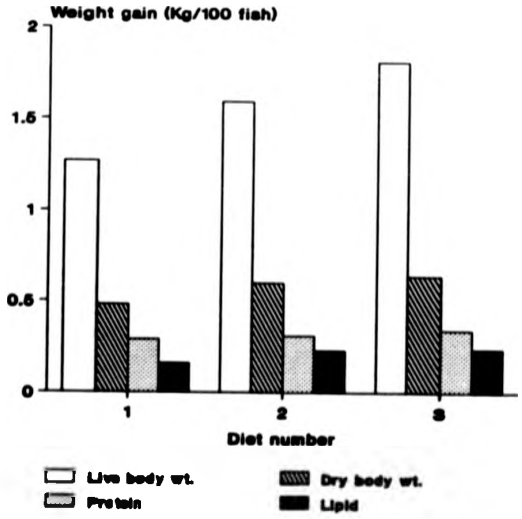

Weight gain (Kg/100 fish)
2
1.5
1
0.5
0
1
2
3
Diet number
Live body wt.
Dry body wt.
Protein
Lipid

Table 32 shows apparent digestibility coefficients (ADC) of dry matter, nutrients and gross energy for the experimental diets when crude fibre was used as inert marker for calculations. The ADC of protein was not affected neither by the lipid nor the fibre levels in the diets. Lipid digestibility increased as dietary lipid increased from 10.75% to 14.15%, but declined when the lipid level was 16.27%. This effect is interpreted as due to the increasing amount of fibre in the highest lipid diet. Similarly, the ADC of gross energy, dry matter and carbohydrate decreased as the amount of dietary fibre was higher. The negative value of carbohydrate ADC in diet 3 suggests a lower accuracy when calculations were based upon fibre as marker, in comparisson with Cr_2O_3.

5.3.2.2 Gains

The net gains of live and dry body weights, protein and lipid are shown in Table 33 and figure 31. These values were only statistically different for lipid ($p < 0.05$), but in all of them higher lipid levels supported higher net gains.

The conversion of feed and dry matter, protein and lipid of feed are also shown in Table 33. Conversions of feed, dry matter and protein were more efficient as the lipid level increased, protein gain differences being statistically significant ($p < 0.05$). Lipid conversion was best at 14.15% dietary lipid, and very similar at 10.75% and 16.27%.

Table 32. Apparent digestibility coefficients (ADC, %) in experiment VI when crude fibre was used as internal marker for calculations.

	DIET NUMBER 1	2	3
Dry matter (*)	48.4 (87.5)	37.0 (83.2)	32.2 (76.5)
Protein	90.2	90.3	90.4
Lipid	98.9	99.2	96.7
Carbohydrate (*)	(73.7)	(47.9)	(-31.2)
Gross Energy	85.4	82.4	79.0

(*) Re-calculated values in parenthesis (See Table 27).

Table 33. Effect of different dietary lipid levels on weight gain and conversion in *S.aurata* juvenies (*)

DIETARY LIPID (%)	10.75	14.15	16.27
	(Kg gain/100 fish)		
Live body weight	1.27[a]	1.59[a]	1.81[a]
Dry body weight	0.48[a]	0.60[a]	0.64[a]
Protein	0.29[a]	0.31[a]	0.34[a]
Lipid	0.16[a]	0.23[b]	0.24[b]
	(intake/gain ratio)		
Feed	2.16[a]	1.74[a]	1.54[a]
Dry matter	5.72[a]	4.60[a]	4.38[a]
Protein	5.02[a]	4.72[ab]	4.35[b]
Lipid	1.84[a]	1.70[a]	1.86[a]

* Data: Initial body weight : 4.643 Kg/100 fish
Note. Values in the same row with same superscripts are not significantly different ($p < 0.05$)

Table 34. Partition of dietary energy and energy retention efficiences.

DIETARY LIPID LEVEL (%)	DE	RE	ZE+UE	ME	HE	ERE
			(Mj per Kg feed intake)			
10.75	14.08	1.40	0.04	14.04	12.64	0.10
14.15	14.70	4.04	0.04	14.66	10.62	0.27
16.27	14.83	4.82	0.04	14.79	9.97	0.32

See Table 30.

5.3.2.3 Partition of dietary energy and energy retention efficiencies

As shown in Table 34, there were no appreciable differences in non-faecal energy losses, and increased dietary lipid levels supported increased ME, RE and ERE.

5.4 DISCUSSION

Carbohydrates such as fibres are considered undigestible fractions in the feed (N.R.C., 1983), and even omnivorous fish species such as common carp are practically unable to digest crude fibre. Hence the crude fibre content in the diet can be used as an indicator in digestibility studies (Steffens, 1989). When comparing crude fibre with Cr_2O_3 as internal markers in these experiments, despite a certain similarity in trends of results using both substances, the fact that crude fibre content estimates lead to higher errors was apparent in diet 3 (Trial V) and diet 3 (Trial VI). More accurate results seem, therefore, to be obtained with the use of Cr_2O_3. However, the presence of NaCl in faeces and the possible leaching effect previously discussed makes the comparison between markers difficult.

In the first experiment, it is clear from the high protein digestibility values obtained that no appreciable negative effects of antinutrients were shown in any diet. Diet 4, containing 14.1% of wheat bran performed well. This result

opens the possibility of utilizing wheat bran as source of carbohydrate for practical diets in *S.aurata*.

The high digestibility values observed for the fish oil in both experiments demonstrate that this species has the ability to utilize this fat source efficiently.

S.aurata juveniles showed a slightly limited ability to use carbohydrate, digestibility values being lower than 85% regardless of carbohydrate source in the first experiment. This fact has been reported for similar marine carnivorous species (Shimeno *et al.*, 1979; Jobling, 1981), probably because of poor digestibility of polysaccharides. Increased amounts of fibre in the second experiment produced lower carbohydrate digestibility. This has also been reported for *Oreochromis niloticus* and *Chanos chanos* (Wang *et al.*, 1985; Ferraris *et al.*, 1986). It is interesting to observe that there were no deletereous effects in diet 4 (14.1% wheat bran) due to antinutrients.

The dry matter digestibility is the combination of the partial digestibilities of all the materials in the diet. In the first experiment the origin of the carbohydrates affected the ADC of the dry matter. Thus, diets 2 and 1 containing a high percentage of dextrin had the highest digestibility values to dry matter. These were followed by diet 3 in which the only carbohydrate source was corn starch, and diet 4 in which wheat bran was the only source of carbohydrate. The lowest value for diet 4 is probably due to the higher fibre content.

The results of the first experiment seem to indicate that corn starch was the most effective carbohydrate source in terms of "energy-yielding". Red sea bream (*Chrysophrys major*) has also been reported to utilize starch more efficiently than dextrin or glucose (Furuichi and Yone, 1982). Dietary dextrin levels above 10% and 20% produced growth retardation, low feed efficiency and poor carbohydrate digestibility in *Pleuronectes platessa* and *Seriola quinqueradiata*, respectively (Cowey and Sargent, 1972; Furuichi and Yone, 1980).

Increased dietary lipid levels in the second experiment supported increased metabolizable energy (ME), recovered energy (RE), energy retention efficiency (ERE) and better protein conversion, indicating improved utilization of protein and energy. Similar results were reported by Cho (1987), when feeding rainbow trout of 0.7g initial body weight with dietary lipid levels ranging from 13 to 23%.

Very similar lipid conversion ratios with increased dietary lipids indicated that an increased proportion of lipid in the diet was used as fuel, hence the protein conversion was improved. This also may have indicated that high lipid diets did not promote the high carcass lipid deposition as expected, suggesting the protein sparing effect of dietary lipid reported in the previous chapter and for similar fish species (Takeuchi *et al.*, 1978; Takeda *et al.*, 1975; Bromley, 1980).

Pereira *et al.* (1987) reported a beneficial effect on growth and feed utilization in gilthead sea bream as dietary lipid content increased, this was also

reported for other fish species such as *Dicentrarchus labrax* (Metailler *et al.*, 1981), *Anguilla anguilla* (Degani, 1986), *Seriola quinqueradiata* (Shimeno *et al.*, 1980), *Morone saxatilis* (Berger and Halver, 1987) and *Oncorhynchus mykiss* (Watanabe *et al.*, 1979; Alsted and Jokumsen, 1989).

The fact that no differences in non-faecal energy losses were apparent in both experiments may indicate that the protein from the fish meal was utilized to a minimum for energy purposes, since the diets contained enough non-protein "energy-yielding" nutrient (lipid) (Cho, 1987).

6. GENERAL DISCUSSION

6. GENERAL DISCUSSION

Research in fish and shellfish nutrition is a relatively new area. However, aquaculture origins extend back at least three thousand years (Bardach *et al.*, 1972), and most of the world aquaculture practices nowadays are still based on ancient production methods.

In most developed western countries and Japan a series of factors in the middle of the 1960s made clear that there were serious constraints to the future development of traditional fishery industries. A trend towards the intensive cultivation of high-value fish and shellfish resulted, which quickly gave rise to the term aquaculture. Pioneering efforts in fish nutrition were made by American and Japanese investigators in particular (Lovell, 1989; Steffens, 1989).

Not surprisingly, most research concentrated principally on salmonids, which were species of high commercial interest, and the extent to which this research effort has contributed to the massive development of the salmon industry in north-European countries nowadays, for example, cannot be underestimated (Steffens,1989).

The fact that *Sparus aurata*, a Mediterranean species with high economical value, which has been cultured on a commercial scale since the early 1980s, has not been subject to similar research interest can be, at least partially, understood

since most Mediterranean countries suffer a lack of basic and applied research development, especially in aquaculture (O.C.D.E., 1989).

More recently, however, a greater level of resources is being devoted to aquaculture research in some of these Mediterranean countries and more people are engaged with the problems of fish nutrition (Ricard, 1990).

An understanding of dietary energy budgets and efficiency of protein utilization is of greatest importance for successful diet formulation, and this requires systematic and orderly nutritional research applied to every single cultured fish species (Halver, 1976).

The work presented here attempts to address several nutritional aspects of *S. aurata*. Some of these form extensions/improvements to previous works and others study contributory aspects, all closely inter-related.

Although the number of experiments performed are not conclusive, in the present study the dietary protein requirements found for *S. aurata* were 55% and 42% crude protein for fry and juveniles, respectively. These values are in the same range as those found for other marine carnivorous fish species, although Sabaut and Luquet (1973) reported a value of 40% protein for 3g initial body weight individuals of the same species.

Such differences are interpreted as partially produced by the use of different dietary energy levels and protein sources, as well as different environmental conditions. In addition, the analysis of the data on growth and protein gain may lead to significantly different results as a consequence of the use of one of the two different statistical techniques more frequently employed.

Thus, when dose-response analysis, using the "broken-line" technique, is applied to the data on weight gain from this work, the resulting protein requirements are 49.7% and 41.0% for fry and juveniles, respectively (Figures 9 and 15), which are much in agreement with the results of Sabaut and Luquet. Similarly, if data from these authors on weight gain are analysed with the polynomial regression technique, the resulting protein requirement of 3g *S. aurata* fingerlings is 59.5% (Figure 32).

One of the two above techniques has been chosen by many investigators. Some of them showed their preference for the polynomial regression analysis (Cowey *et al.*, 1972; Santiago and Reyes, 1991; Teng *et al.*, 1978; Shi *et al.*, 1988), and others employed the broken-line (De Long *et al.*, 1958; Anderson *et al.*, 1981; Papaparaskeva and Alexis, 1986; Satia, 1974). In this group, it may be reasonable to argue that the use of the polynomial regression technique would have resulted in significantly higher protein requirements for *Oncorhynchus tschawytscha, Micropterus dolomieu* and *M. salmoides, Mugil capito* and *Oncorhynchus mykiss,* respectively.

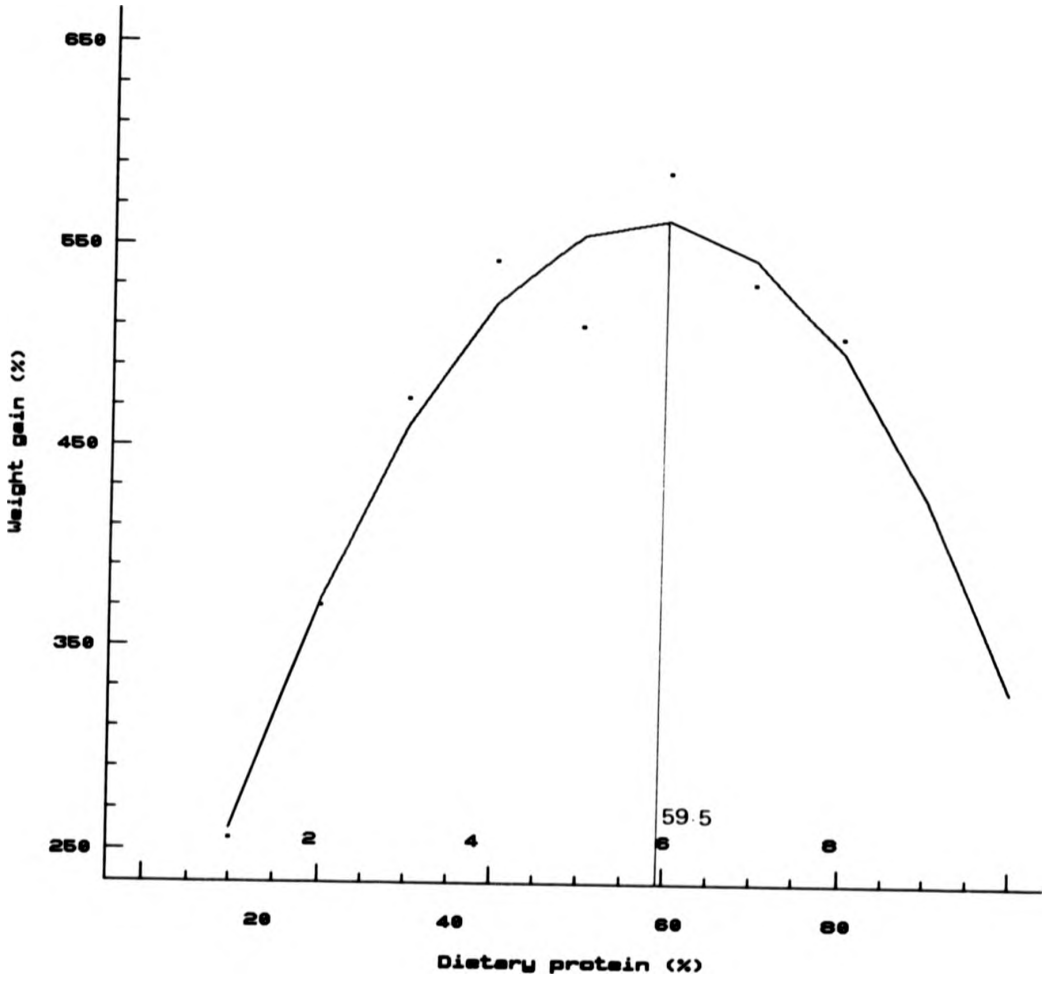


Figure 32. Dose response analysis (polynomial regression) of data on weight gain (%) against different dietary protein levels from the work of Sabaut and Luquet (1973).

Other authors prefer either to report the economically optimal dietary protein resulting from the polynomial analysis (De Silva and Gunasekera, 1991), or to make a selection from the two performed analyses (Dabrowski, 1977).

The above suggests that a new source of difficulty when comparing results from different authors should be added to the list already mentioned, underlining the necessity to standardize experimental approaches. The results of this work suggest that the choice of the analysis technique should be based on the better fit to fish response, although the broken-line and polynomial models applied to the experimental data proved to be inappropriate.

Higher carbohydrate content in low protein diets produced increased liver glycogen accumulation, suggesting a limited ability of *S.aurata* to utilize dietary carbohydrate.

The results from the experiments on the sparing effect of dietary lipids on protein in *S. aurata* fingerlings and growers showed that the optimum dietary protein levels for both sizes were about 52% and 53%, respectively (Section 4). The requirements for protein of growers were slightly higher than expected, but overall confirmed the results from the first experimental chapter.

Thus, the optimum dietary protein level found for 0.8g fry was 55%, a value which in the case of 5g fingerlings was 52%. However, with 90g sea bream growers, best performance was observed with 53% protein, while optimum dietary protein level for 60g juveniles was 42%. The decrease of protein

requirements in juveniles when compared with fry and fingerlings agrees with the well documented fact that smaller fish require higher levels of protein than larger fish (Section 3.4). However, optimum dietary protein levels for growers could have been expected in the same range as that of juveniles. A possible explanation could be a higher protein demand during sexual ripening of growers for gonad development. Unfortunately, this factor was not taken into account in the present experiments, and further research work will be needed to confirm or disprove this theory.

The protein sparing effect of dietary lipids was more evident with fingerlings, where reduction of dietary protein from 52% to 46% and increase of lipids from 9% to 15% in the diets resulted in better performance of fish. Then, best protein to energy ratios (P:E) were 21.9 and 26.4 g protein/Mj of gross energy for fingerlings and growers, respectively, and for a water temperature range of 20-22°C. These values were in the range of results reported for other fish species (Table 2), and suggest that an increase of dietary lipids usually results in an improved growth and food conversion efficiency, especially in carnivorous fish species (Alliot *et al.*, 1979; Shimeno *et al.*, 1980; Degani, 1986; Berger and Halver, 1987; Alsted and Jokumsen, 1989).

Accordingly with these results, proportions of dietary protein and lipids of 45% and 15%, respectively, can be proposed for *S.aurata* fingerling diets, and it is suggested that dietary protein level could also be reduced from 55 to 50% in diets for fry, providing lipid levels are increased from 8 to 15%.

The lack of conclusive results in the experiment with growers gives little room to suggest any protein-sparing effect in juveniles. However, this effect was apparent in experiment VI, when increased amounts of dietary lipids improved overall dietary protein and energy utilization.

The increase of dietary lipids produced an increment in carcass lipid deposition, which took place both in viscera and non-visceral tissues (Table 20), but these levels were in all cases well below reported carcass lipid contents in wild *S. aurata* in the Mediterranean (Section 4.4). The latter suggests that there is still scope for further increase in high-quality lipids in formulated diets for this species, and that more research is needed to determine if higher reductions in dietary protein can be achieved by this means.

The inclusion of higher levels of dietary lipids provoked a decrease in liver lipid content, which could be due to a reduction in liver fatty acid synthesis, as enough fat for intermediary metabolism was already present. The energy otherwise required for this process could then be used for other metabolic purposes and hence improve protein utilization (Sanchez-Muros, 1990).

Differences between the results of the present study and previously reported results for *S. aurata* from other workers are explained by poor utilization of purified diets and vegetable oils by this species and different feeding rates and environmental conditions used by other authors (Sabaut and

Luquet, 1973; Marais and Kissil, 1979; Kissil and Groop, 1984). The use of non-isoenergetic diets and restrictive variable ranges can also be argued.

There is, however, an agreement with other authors (Pereira *et al.*, 1987; Takeuchi *et al.*, 1991) when similar feed ingredients to those employed in this work were used with *S.aurata* and *C.major*, in the fact that high energy and high protein diets improved fish performance.

In the digestibility experiments, several techniques and procedures employed are suggested as the source of errors when calculating apparent and total digestibility coefficients (Cho, personal communication, 1991).

In the first place, faeces break-down could have been diminished by using higher slopes in the settling columns and by collecting faeces on a continuous basis, as they were settled, in centrifugal tubes.

The estimation of carbohydrate contents in faeces should have included determination of NaCl levels when calculations were made by difference, and biochemical analysis (especially for carbohydrate) would have produced more accurate results.

In general, a margin of error must be assumed in absolute values of nutrient digestibility obtained after re-calculations, although relative values between different treatments are consistent with those obtained for weight gains, conversions and energy partition.

Thus, having in mind the above reservations, some valuable conclusions may be drawn when comparing diets in these experiments.

The use of crude fibre as an internal marker to calculate nutrient digestibilities resulted in less accurate results than those obtained when Cr_2O_3 was used as reference marker. However, the fact that similar trends in ADC results were present suggests that crude fibre could be an appropriate internal marker for digestibility studies when problems such as faeces break-down and NaCl estimations are overcome.

Low carbohydrate digestibility values shown by *S. aurata* juveniles, regardless of carbohydrate source, seem to indicate a limited ability to use this nutrient, as could be expected from data obtained with other marine carnivorous fish species (Shimeno et al., 1979; Jobling, 1981). However, protein and lipid digestibilities were always above 94% and 99%, respectively. No appreciable negative effects of antinutrients were shown in any diet, and the high ADC values observed for the fish oil in both experiments demonstrate that this species has the ability to utilize this fat source efficiently.

Increased amounts of fibre in diets produced lower protein and lipid digestibility, this effect being more pronounced on carbohydrate digestibility. This has also been reported for other fish species (Wang *et al.*, 1985; Ferraris *et al.*, 1986).

The results of the first digestibility experiment also suggest that corn starch was the most efficient carbohydrate source as "energy-yielding" for *S.aurata*, as similarly reported for red sea bream (Furuichi and Yone, 1982).

As a consequence, practical diets for *S. aurata* should contain carbohydrate and fibre levels not higher than 20% and 6%, respectively.

Wheat bran, which was also used as carbohydrate source in a previous experiment with *S. aurata* growers, showed reasonably good results in this experiment, indicating that this product, locally available, can be a useful ingredient if the prospects to commercially produce practical diets for this species are considered in Canary Islands.

When comparing different energy levels in diets, increased amounts of dietary lipids improved overall dietary protein and energy utilization, also suggesting the protein-sparing effect of dietary lipids. These beneficial effects of increased dietary lipid contents agree with those reported by Cho (1987) with rainbow trout.

The studies reported suggest that *S. aurata* juveniles require 35.7 KJ digestible energy per gram of digestible crude protein intake, for a diet containing 48% digestible crude protein and 15.8% digestible lipid.

7. CONCLUSIONS

Based on the preliminary findings of this thesis a list of **conclusions** may be summarized as follows:

1. The dietary protein requirements found for *S. aurata* fry and juveniles were 55% and 42%, respectively.

2. The differences found with results from other authors are interpreted mainly as a consequence of the use of different techniques for analysing experimental data, underlining the need for standardization of experimental methods in order to obtain comparable results. In addition, the use of different dietary ingredients, feeding rates and environmental conditions by other authors contributes to explanation of different results.

3. There is a need for a series of experiments on dietary protein substitution in order to reduce production costs of high protein diets for *S. aurata*.

4. The dietary protein requirements found for *S. aurata* fingerlings and growers were 52% and 54%, respectively, and this level of protein could be reduced to 45% when dietary lipid increased from 9% to 15%. This protein-sparing effect was more pronounced in smaller fish.

5. The above results suggest that protein levels similarly could be reduced to 50% for fry, providing an increment in dietary lipid levels from 9% to 15-17%.

6. Excess or reserve lipids are stored both in visceral and non-visceral tissues in *S. aurata*.

7. Lipid carcass deposition was incremented as dietary lipid increased, although levels were not excessive when compared to wild animals.

8. The above findings may suggest that there exists scope for further dietary protein substitution by lipids. This should be studied in future experiments with *S. aurata*.

9. Optimum dietary protein requirements found for *S. aurata* growers could have been expected in the same range as that of juveniles (42%). A possible explanation for the high values obtained (52%) could be a higher protein demand during sexual ripening for gonad development. Further research will be needed to test this hypothesis.

10. Some techniques and procedures used when determining nutrient digestibilities were identified as sources of errors. Improvements are discussed.

11. The use of crude fibre as internal marker for digestibility studies was less accurate than when Cr_2O_3 was employed.

12. A limited ability of *S. aurata* juveniles to use dietary carbohydrate, as well as a negative effect of increased levels of fibre in the diets, may indicate that formulated practical diets for this species should include maximum fibre and carbohydrate levels of 6% and 20%, respectively.

13. Dietary proteins and lipids were well digested, underlining their importance as "tissue-yielding" and "energy-yielding" nutrients for this species.

14. Further digestibility studies should be carried out with smaller fish sizes to compare nutrient digestibility at different life cycle stages.

15. Corn starch was the most effective carbohydrate source in terms of "energy-yielding", although wheat bran appeared to be a suitable carbohydrate source for practical diets.

16. A wider range of carbohydrate sources and feedstuffs must be assayed in future experiments with this fish species in order to assess their suitability for commercial diets.

17. Increased dietary lipid levels supported improved dietary protein and energy utilization, underlining the importance of this nutrient as "energy-yielding" for *S. aurata*.

18. The method of estimating ME, ZE + UE, HE and nutrient and energy

retention efficiencies using comparative carcass analysis proposed by Cho (1987) resulted in a practical and reliable tool for evaluation of fish diets.

19. Suggested nutrient levels in practical diets for *S. aurata* can be summarized as follows (% dry wt.):

Nutrient	Fry From weaning up to 2g	Juvenile 2g-60g	Grower 60g →
Protein (From fish meal)	50	45	45
Lipid (From fish oil)	17	15	13
Carbohydrate (From wheat bran)	20	20	20
Fibre	6	6	6
Gross Energy (MJ/Kg)	21.3	19.4	18.7
P:E ratio (g/MJ of GE)	23.5	23.2	24.1

In conclusion, it can be seen that much more research is needed to provide a sound knowledge of the nutritional requirements of the gilthead sea bream in order to provide a solid support to the commercial culture of this species. It is hoped that this thesis may provide guidelines and stimulus for further research aimed at regional aquaculture development in the Canary Islands.

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8. REFERENCES

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APPENDIX I

APPENDIX I.

Calculation of essential amino acid (EAA) requirements of *S.aurata* based on the carcass analysis method, and amount of amino acids supplied in diets containing 40% and 55% protein.

This method is based on the direct correlation found between the pattern of EAA in fish tissues as a percentage of the total EAA and the dietary requirements for EAA (Cowey and Tacon, 1981; Tacon, 1990).

Dietary EAA requirements can be initially computed on the basis of the carcass EAA pattern present within 35% of the known dietary protein requirements of the majority of farmed fish species. On a general basis EAAs (including the non-essential cystine and tyrosine) constitute about 35% of the total dietary protein required by fish. Thus, for a particular fish species known to have a dietary protein requirement of 40%, then dietary EAA requirements would be computed on a carcass EAA pattern of 35% of the dietary protein level.

In this case (Table 5a, Section 2.4), the carcass EAA pattern for arginine is 12.71% of the total EAA plus cystine and tyrosine present, then dietary requirement level for arginine would be:

$$\frac{40 \times 35 \times 12.71}{10000}$$ or 1.78% of the dry diet.

Thus, based on raw data on carcass EAA analysis (g AA/100g prot), Table 35a shows estimated EAA requirements for diets containing 40% and 55% protein, as percentage of dry diet (These data are summarized in Table 5a, Section 2.4).

Similarly, based on the amino acid profile of the sardine meal (*S.pilchardus*) utilized as sole food, the amount of EAA supplied in diets containing 40% and 55% protein were calculated (Table 35b).(These data are summarized in Table 5b, Section 2.4).

Table 35a. Carcass essential amino acid (EAA) profile of *S.aurata*. A) g AA/100g prot. B) EAA as a percentage of the total EAA in carcass. C) EAA in grams per 100g of dry diet (40% prot.). D) EAA in grams per 100g of dry diet (55% prot.)

	A	B	C	D
Arginine	6.1317	12.40	1.74	2.39
Histidine	2.5848	5.23	0.73	1.00
Isoleucine	5.3081	10.74	1.50	2.07
Leucine	7.4209	15.01	2.10	2.89
Lysine	7.7622	15.70	2.20	3.02
Methionine	2.8238	5.71	0.80	1.10
Phenylalanine	3.5612	7.21	1.01	1.39
Threonine	5.1999	10.52	1.47	2.03
Valine	4.6229	9.35	1.31	1.80
Cystine *	0.9907	2.00	0.28	0.38
Tyrosine	3.0198	6.11	0.85	1.18

* Non-essential amino acids

Table 35b. EAA profile of the sardine meal utilized as sole food: A) g AA/100g prot. B) % of the total EAA. Amount of EAA supplied in diets containing:C) 40% protein (EAA in grams per 100 g of dry diet), and D) 55% protein (EAA in grams per 100g of dry diet).

	A	B	C	D
Arginine	5.8674	10.17	2.35	3.23
Histidine	3.4123	5.92	1.36	1.88
Isoleucine	6.7471	11.70	2.70	3.71
Leucine	8.6317	14.96	3.45	4.75
Lysine	8.9880	15.58	3.60	4.94
Methionine	3.0149	5.23	1.21	1.66
Phenylalanine	4.6774	8.11	1.87	2.57
Threonine	5.9917	10.39	2.40	3.30
Valine	5.5558	9.63	2.22	3.06
Cystine *	1.1519	1.99	0.46	0.63
Tyrosine *	3.6484	6.32	1.46	2.01

* Non-essential amino acids