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Factorial Approach to Determining Energy and Protein Requirements of Gilthead seabream (*Sparus aurata*) for Optimal Efficiency of Production

I n a u g u r a l - D i s s e r t a t i o n

zur
Erlangung des Grades
Doktor der Agrarwissenschaften
(Dr. agr.)

der
Hohen Landwirtschaftlichen Fakultät
der
Rheinischen Friedrich-Wilhelms-Universität
zu Bonn

vorgelegt am 15. Dezember 2003

von
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Tag der mündlichen Prüfung: 16. Juni 2004

Gedruckt bei:

Faktorielle Berechnung des Energie- und Proteinbedarfs von Goldbrassen (*Sparus aurata*) zur Optimierung der praktischen Fütterung.

Ingrid Lupatsch

Der Bedarf wachsender Fische an Energie und Protein im Futter läßt sich mit Hilfe der faktoriellen Methode quantifizieren, die den Bedarf als Summe aus Wachstum und Erhaltung unterstellt. Die Aufnahme über das Futter kann daher unter Verwendung der jeweiligen partiellen Wirkungsgrade kalkuliert werden.

Das Wachstum von Goldbrassen wurde als Funktion von Körpergewicht und Temperatur mit Hilfe der folgenden Gleichung berechnet: $y = 0{,}92 \cdot BW(kg)^{0{,}613} \cdot e^{0{,}065 \cdot T}$ (mit y = Gewichtszunahme in g $Fisch^{-1}$ Tag^{-1}, BW = Körpergewicht in kg und T = Wassertemperatur in °C). Die Zusammensetzung des Gewichtszuwachses wurde durch Ganzkörperanalysen von Fischen im Gewichtsabschnitt von 1 bis 420 g bestimmt. Der Energiegehalt hing vom Gewicht des Fisches ab und stieg von 5,0 auf 11,0 MJ je kg Körpermasse an, während der Proteingehalt mit 177 g je kg konstant blieb. Mit Hilfe der vergleichenden Schlachttechnik wurde der Gewichtsverlust der Fische während einer Hungerperiode bestimmt. Die Werte betrugen 42,5 kJ $BW(kg)^{-0{,}83}$ Tag^{-1} für Energie, beziehungsweise 0,42 g $BW(kg)^{-0{,}70}$ Tag^{-1} für Protein.

Die Wirkungsgrade für Erhaltung und Wachstum wurden für verdauliche Energie (DE) und verdauliches Protein (DP) bestimmt, indem Goldbrassen von zwei unterschiedlichen Größen zunehmende Futtermengen erhielten und zwar von Null bis zur maximalen freiwilligen Futteraufnahme. Zur Schätzung der optimalen Proteinausnutzung für den Proteinansatz erhielten Goldbrassen in drei aufeinanderfolgenden Versuchen Futter mit unterschiedlichem DCP/DE - Verhältnis (34-15 g je MJ). Die Fische wurden per Hand bis zur offensichtlichen Sättigung gefüttert und der anschließende Energie- und Proteinansatz gemessen.

Es wurde ein Erhaltungsbedarf an verdaulicher Energie in Höhe von 53,0 kJ BW $(kg)^{-0{,}83}$ Tag^{-1}, sowie an verdaulichem Protein in Höhe von 0,77g $BW(kg)^{-0{,}70}$ Tag^{-1} ermittelt. Die Beziehung zwischen der Aufnahme an DE und Energieansatz erwies sich als konstant mit einem Wert von $k_{DEg} = 0{,}56$ und war unabhängig von Futteraufnahme, Körpergewicht und DCP/DE-Verhältnis. Die Effizienz der Proteinausnutzung variierte zwischen 0,33 und 0,56 in Abhängigkeit vom DCP/DE-Verhältnis in der Ration. Die optimale Proteinausnutzung für Proteinansatz wurde als $k_P = 0{,}48$ errechnet. Die Verwendung dieser Werte ermöglicht eine Optimierung der Fütterungstabellen für die praktische Fütterung bei der Haltung von Goldbrassen.

Factorial Approach to Determining Energy and Protein Requirements of Gilthead Seabream (*Sparus aurata*) for Optimal Efficiency of Production

Ingrid Lupatsch

Requirements for dietary energy and protein in growing fish can be quantified using the factorial approach which assumes that the requirement is the sum of growth and maintenance. Thus dietary intake can be calculated using the respective partial efficiencies of utilization.
Growth for gilthead seabream as a function of body weight and temperature was predicted by the equation: $y = 0.92 \cdot BW\ (kg)^{0.613} \cdot e^{0.065 \cdot T}$ (where y = weight gain in g $fish^{-1}$ day^{-1}, BW = body weight in kg and T = temperature in ^{0}C). The composition of the gain was measured by analyzing whole fish ranging from 1 to 420g. The energy content was dependent upon fish weight and increased from 5.0 to 11.0 MJ kg^{-1} body mass, whereas the protein content remained constant at 177g kg^{-1}. The comparative slaughter technique was used to determine the loss in the fish during starvation and the values amounted to 42.5 kJ $BW(kg)^{-0.83}$ day^{-1} and 0.42g $BW(kg)^{-0.70}$ day^{-1} for energy and protein respectively.
The efficiencies of utilization of digestible energy (DE) and digestible protein (DP) for maintenance and growth were determined by feeding seabream of two sizes at increasing feeding levels, from zero to maximum voluntary feed intake. To estimate optimal protein utilization for protein deposition seabream were fed diets formulated to contain varying DCP/DE ratios (34 - 15g MJ^{-1}) in three consecutive trials. Fish were hand-fed to apparent satiation and the subsequent energy and protein gain were measured.
The requirement for digestible energy for maintenance was determined to be 53.0 kJ $BW(kg)^{-0.83}$ day^{-1} and for digestible protein 0.77g $BW(kg)^{-0.70}$ day^{-1}. The relationship between DE intake and energy gain was found to be constant at a value of $k_{DEg} = 0.56$ and was independent of feed intake, body weight and DCP/DE ratio. Efficiency of protein utilization for growth varied between 0.33 and 0.56 depending on the DCP/DE ratio in the diet and optimal protein utilization for protein deposition was estimated to be $k_P = 0.48$. Using these values allows optimization of practical feeding tables for seabream culture.

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Abbreviations

ADC	apparent digestibility coefficient
BW	body weight
CP_{starv}	protein loss during starvation
CP	crude protein
CL	crude lipid
CC	crude carbohydrate (= organic matter - crude protein - crude lipid)
DCP	digestible crude protein
DCP_m	DCP required for maintenance - zero protein growth
DCP_g	DCP required for growth above maintenance
DE	digestible energy
DE_m	DE required for maintenance - zero energy growth
DE_g	DE required for growth above maintenance
DM	dry matter
E_{starv}	energy loss during starvation
FCE	feed conversion efficiency
FCR	feed conversion ratio
GE	gross energy
k_{DCPm}	efficiency of utilisation of DCP below and at maintenance
k_{DCPg}	efficiency of utilisation of DCP above maintenance
k_{DCPtot}	efficiency of utilisation of DCP for maintenance and growth
k_{DEm}	efficiency of utilisation of DE below and at maintenance
k_{DEg}	efficiency of utilisation of DE for growth
k_{DEtot}	efficiency of utilisation of DE for maintenance and growth
k_P	energy requirement for protein deposition
k_L	energy requirement for lipid deposition
MBW	metabolic body weight
n	number of measurements
OM	organic matter
RE	retained energy
RCP	retained CP
SD	standard deviation
SE	standard error
SGR	specific growth rate
r^2	coefficient of determination
T	temperature
WW	wet weight

Definitions

Geometric mean weight	=	$(\text{initial BW} \cdot \text{final BW})^{0.5}$
Feed conversion ratio	=	feed intake (g DM) / weight gain (g WW)
Feed conversion efficiency	=	weight gain (g WW) / feed intake (g DM)
Specific growth rate	=	$[\ln (BW)_2 - \ln (BW)_1]$ / days of growth

Common and Latin names for fish species appearing in text

African catfish	*Clarias gariepinus*
Atlantic salmon	*Salmo salar*
Atlantic cod	*Gadus morhua*
Common carp	*Cyprinus carpio*
Channel catfish	*Ictalurus punctatus*
European seabass	*Dicentrarchus labrax*
Gilthead seabream	*Sparus aurata*
Rainbow trout	*Oncorhynchus mykiss*
Red drum	*Sciaenops ocellatus*
Red seabream	*Pagrus major*
Tilapia	*Oreochromis niloticus*
Turbot	*Psetta maxima*
Yellowtail	*Seriola quinqueradiata*

1 Introduction

Depletion of marine aquatic sources, caused by over-fishing, environmental pollution and the growing demand for fish and other aquatic organisms, has stimulated the development of aquaculture in marine and inland waters all over the world. Aquaculture represents one of the fastest growing food producing sectors in agriculture, and it is continuing to grow. World wide, this sector has increased at an average rate of 9.2 percent per year since 1970, compared with only 1.4 percent for capture fisheries and 2.8 percent for terrestrial farmed meat production systems.

According to the Food and Agriculture Organisation of the United Nations (FAO) aquaculture's contribution to global supplies of fish increased from 3.9 percent of total production by weight in 1970 to 27.3 percent in 2000 (FAO 2002). Although marine capture fisheries still provide for a major portion of the food supply, the possibilities of meeting the increasing demands for seafood through capture are now recognised to be limited (Naylor *et al*. 1998, 2000; Tidwell and Allen 2001; Pauly *et al*. 2002). Thus, while capture fisheries are confronted with major problems of limited supply and potential for growth, aquaculture is a growing industry capable of meeting the increased demands for high quality food.

In production terms the Mediterranean fish farming industry has been a spectacular success in less than two decades, equal to that of salmon farming. One of the major species of interest is gilthead seabream (*Sparus aurata*). Gilthead seabream is found in the Mediterranean and Black Seas and extends into the Atlantic Ocean from the British Isles south to Senegal. Farming of seabream is carried out mainly under intensive pond and cage culture in the Mediterranean Sea. Most countries around the Mediterranean culture seabream, Greece, Turkey and Spain are the main producers in the region, accounting for over 70% of the production. Production went up from an estimated 121 tons of fish in 1985 to 25 700 tons in 1995 and up to 69 750 tons in 2001 (FEAP 2002).

In Israel the production of marine fish grew rapidly only during the last decade. Mariculture is relatively new to the local aquaculture industry and production of marine fish grew from 400 tons in 1994 to an estimated 2700 tons in 2000 (Snovsky and Shapiro 2000). The main production of seabream is taking place in cages in the Gulf of Aqaba in the Red Sea. The farms are situated in southern Israel in close proximity to the borders of Egypt, Jordan and Saudi Arabia. The water in this region is highly oligotrophic (Reiss and Hottinger 1984) due to minimal runoff from the surrounding deserts and a low population density. The high nitrogen and phosphorus concentrations of aquaculture discharges could be a potential threat to the oligotrophic water of the Gulf. Nutrient enrichment can alter the community of plants and organisms (Parsons *et al*. 1977) and might endanger fragile ecosystems like the coral reefs (Bell *et al*. 1989), which are abundant in the Gulf. There is growing pressure here as anywhere in the Mediterranean to develop methods for predicting the effects of fish farms on their surroundings and to develop environmentally friendly mariculture. The Mediterranean coast, which is about 46,000 km long and is highly populated, displays a wide range of geographical characteristics and supports many functions, such as tourism, residential development, and conservation, which may compete with aquaculture for resources.

A shift to more intensive aquaculture practices, made possible by the availability of better, formulated diets, has been partly responsible for the increase in aquaculture. Considerable progress has been made in the last years in the study of the dietary nutrient requirements of fishes, mainly salmonids. Despite some obvious similarities between fish and other vertebrates in basic qualitative nutrient needs such as energy, essential amino acids, fatty acids, vitamins and minerals there seem to be differences in quantitative requirements mainly for energy and protein. For example the optimal dietary protein level required for farmed fish is reported to be much higher and is in the range of 30 to 50% of the diet.

Many problems are encountered when feeding fish, much more so than with feeding domestic animals. Delivery of feed to fish in a water medium requires particular physical properties of feed together with special feeding techniques. It is not advisable to feed fish on an *ad libitum* basis, as it is done with most farm animals, since feed that is not consumed or not available to the fish will be lost to the surroundings and will result in nutrient enrichment of the water body. Therefore, and due to strict governmental regulations on aquaculture, the trend has been to lower nitrogen and phosphorus levels in the feeds, especially in Scandinavia (Kiaeskou 1991; Enell 1995). The development of high energy diets as another way to reduce aquaculture waste has been reported by several authors (Johnsen and Wandsvik 1991; Alsted 1991; Cho *et al*. 1994). As an example, the digestible energy content in the feed for salmonids increased from 14.8 MJ kg^{-1} in 1975 to 19.2 MJ kg^{-1} in 1989, and the lipid content from 8 to 30% (Johnsen and Wandsvik 1991). At the same time the feed changed from steam pelleted to extruded feed.

As feed is one of the principle costs in fish production, formulation must be based on sound knowledge of nutritional requirements for it to be economical, in addition to environmental concerns. Therefore, feeding models to supply precisely the energy and protein for each fish species to realise its full growth potential are essential in fish farming. Despite the introduction of feeding charts based on nutritional bioenergetics for rainbow trout (Cho and Kaushik 1990; Cho 1992) most fish culture, especially for the major marine species in the Mediterranean, gilthead seabream and European seabass, is practised using provisional feeding regimes. Kaushik (1998) proposed to apply the same bioenergetic principles developed for salmonids to other fish species, but information concerning the prediction of

growth and digestible energy needs are still lacking for most of the marine warm-water species.
Nutrient requirements are generally defined for animals of a given age and for a specific physiological function, such as maintenance, growth, reproduction or production. In fish farming growth of fish flesh is one of the major goals of production. Nutrient requirements in fish are often quantified by dose-response relationships, where diets containing graded levels of a nutrient are fed and the resulting growth is measured. The quantitative requirement for the nutrient is then considered at the level, below which the growth will be depressed or above which it will not increase (Zeitoun *et al.* 1976; Robbins *et al.* 1979; Mercer 1982). These methods are, however, time consuming and limited in their broad application (Baker 1986).

Factorial modelling for estimating requirements has been used in classical animal nutrition for many years but has rarely been applied to fish (Pfeffer and Pieper 1979; Kirchgessner *et al.* 1984; Shearer 1995; Rodehutscord and Pfeffer 1999). Requirements for energy and protein in growing fish can be quantified from the sum of the amounts of energy and protein retained as growth plus the amount of the same nutrients simultaneously lost from the body.
The metabolic expenditure for maintenance is - at a constant temperature - mainly a function of the body weight of the fish. This relationship can be defined by an exponent, which determines the change in the metabolic rate as a function of body weight. The requirement for growth on the other hand is dependent on the amount and composition of the added gain.
The actual requirement for dietary gross energy and protein must take into account the partial efficiency of utilisation of these nutrients for maintenance and for growth. The consequence of the factorial approach is, that protein and energy requirements are not expressed as percentage of the diet, but in terms of absolute daily feed intake per unit of weight and weight gain.

The following equation specifies a formal approach to those calculations:
Requirement $= M \bullet BW\ (kg)^b + G \bullet$ growth
$BW\ (kg)^b$ = Metabolic body weight
M = maintenance requirement
G = coefficient describing the efficiency of utilisation of dietary energy or protein for growth.
This study demonstrates the derivation of the parameters of the factorial model to quantify the daily energy and protein needs in growing gilthead seabream.

A considerate part of this work has been published beforehand and appears in peer reviewed journals (Lupatsch *et al*. 1997; Lupatsch *et al*. 1998 and Lupatsch *et al*. 2001).

2 Common Methodologies

2.1 Experimental system and fish

Gilthead seabream spawned and raised at the National Center for Mariculture (NCM) were used for all the trials in this study. The local broodstock was established some 20 years ago from wild-caught juvenile gilthead seabream from the Bardawil lagoon on the Sinai coast of the Mediterranean Sea. The gilthead seabream, a member of the family Sparidae, a protandrous hermaphrodite, develops as a functional male in its first spawning season, then a certain proportion of the population undergo sex reversal to functional females. Seabream reproduction is controlled by light (or day to night ratio), thermal and social cues. However fish respond primarily to a shortening of the day's length and secondarily to a reduction in water temperature. This strong influence of the varying day length on seabream reproduction makes the fish susceptible to manipulation of reproductive season, which enables year-round egg production in captivity.

All the trials were done at the department for fish nutrition at NCM. The experimental system for growth trials consists of 48 white fibreglass round tanks of each 200L, and additional 6 tanks of each $3m^3$. For digestibility trials 18 square tanks of 400L are available. The tanks are situated outdoors and shaded from direct sunlight by an overhead roof as well as covered with nets to prevent fish from jumping out due to disturbances from the surroundings. Each tank has its own water and air supply. Sea water (41‰ salinity) is pumped from the nearby shore and reaches the tanks at temperatures of 20 to 26°C during the course of the year. Prior to all the experiments, fish were fed a local commercial seabream diet (Matmor Inc., M.P. Evtach, Israel) containing 460g crude protein, 120g crude lipid and 19.6MJ GE per kg feed according to feeding tables developed at NCM. Before each growth experiment, fish were size graded before stocking.

2.2 Diet preparation

All the experimental diets were formulated at the institute, mixed thoroughly in a 25L batch mixer and pelleted using a laboratory model California Pellet Mill with a steam pre-treatment unit. After air drying and when fed to the fish the feeds maintained a residual moisture content of 80 to 90g kg^{-1} diet. Vitamin and mineral mixes as used at the local feed mill (Matmor Inc.) for marine fish were incorporated in the diets.
Vitamin mix at an inclusion level of 10g provided the following per kg of diet: Vitamin A 16000 IU, vitamin D_3 1900 IU, vitamin E 150mg, thiamine 30mg, riboflavin 45mg, niacin

15mg, Ca-pantothenate 30mg, pyridoxine 5mg, folic acid 11mg, vitamin B_{12} 0,12mg, vitamin K 11mg, biotin 0.25mg, inositol 150mg, ascorbic acid 500mg and choline chloride 3g. Mineral premix at an inclusion of 10g supplied per kg diet: MgO 2.5g, KI 1.8mg, $CoCO_3$ 0.66mg, MnO 73.5mg, ZnO 75mg, $CuCO_3$ 57.5mg, $FeCO_3$ 255mg, KCL 3.6g, Na_2SeO_3 0.4mg .

2.3 Sample preparation

Fish sampled for analyses were sacrificed by immersing them into ~4°C cold ice water and stored at -20°C immediately afterwards. Before analysis fish were cut up into small pieces while still frozen and ground twice using a meat grinder fitted with a 3mm die. Samples for estimation of dry matter were taken from the ground fish before the remaining homogenate was freeze-dried. The freeze-dried samples were again mixed in a blender before all remaining analyses. The feed samples were finely ground in a hammer mill using a 1 mm screen.

2.4 Analytical procedures

All analyses were carried out in duplicate, and a deviation of more than 2.5% of the mean was considered unacceptable. Dry matter of feed was calculated by weight loss after 24 h drying at 105°C. Gross energy content was measured by combustion in a Parr bomb calorimeter using benzoic acid as the standard. Crude protein was measured using the Kjeldahl technique and multiplying N by 6.25. Crude lipid was measured after chloroform-methanol extraction (Folch *et al.* 1957). Samples were homogenised with a high speed homogeniser for 5 min and lipid was determined gravimetrically after separation and vacuum drying. Ash was calculated from the weight loss after incineration of the samples for 24 h at 550°C in a muffle furnace.

2.5 Statistical analysis

Mean values and standard deviations were calculated where three replicate tanks were used. When only two replicates were used ranges are given. Fish were stocked in numbers of 18 to 25 per tank and weight gain as well as feed intake data are based on total biomass in each tank. Therefore the tank of fish and not the individual animal is the unit of observation for all the trials. For enhanced clarity the points of interest in graphs are depicted, where appropriate, as average values per treatment (mean ± SD), whereas all the data points were used to establish the equations.

3 Growth and feed intake

3.1 Introduction

A prerequisite for estimating feed requirements is accurate prediction of the growth potential of a fish species. Since 'optimal growth' is generally used as the criterion for estimating the dietary energy and protein requirements, the fish should be fed as close as possible to its maximum capacity at optimal water quality parameters. In view of this, we have to assume that different fish have a genetically determined asymptotic body size and that they are capable of adjusting their feed or energy intake to realise their genetic potential. Therefore, one of the first steps was to establish a workable growth prediction for various water temperatures, as well as the voluntary feed intake for gilthead seabream. Feeds can then be formulated and feeding tables established which are based on daily requirements for energy and protein dependent on anticipated growth.

3.2 Material and Methods

3.2.1 Growth and feed intake

To describe the potential daily weight gain of gilthead seabream data sets were established derived from a total of thirteen growth experiments that were performed with fish ranging from 1 to 400g (Table A1). According to the increasing fish sizes 0.200 or 3 m^3 tanks were stocked at densities ranging from 0.25 to about 10 kg m^{-3} at the end of a growing trial All the feeding trials were carried out at the nutrition unit of the NCM, but only those growth data were utilised, where the commercial seabream diet (Matmor Inc., M.P. Evtach, Israel) was fed as a control. Furthermore, only those growth trials were considered, where feed intake was not limiting. The fish were fed daily to apparent satiation, but taking care that no food was left uneaten. Fish in the growth trials were weighed every 14 days, and the average daily weight gain and daily feed intake between two successive weighings was calculated. The corresponding body weight used in these calculations was the geometric mean weight of the fish during that period. Two sets of 92 data each were thus obtained for daily weight gain and feed intake at different fish weights and water temperatures (detailed description in Table A1, attachment).

All the allometric equations were obtained by applying linear regression analysis to the logarithmic transformation of the data in the form of

$\ln y = \ln a + b \ln x$

The antilog of this expression produces the final equation

$y = a x^{b}$.

With water temperature (T) as the additional variable the equation is as follows:

$\ln y = \ln a + b \cdot \ln x + c \cdot T$

with the final equation of

$y = a x^{b} \cdot e^{c \cdot T}$

Where y describes weight gain and x body weight (BW), this equation can be integrated as shown below and the weight BW_t after t days can be predicted starting from BW_0:

$$BW_t = [BW_0^{c1} + c_2 \cdot e^{c3 \cdot T} \cdot days]^{c4}$$

$c_1 = (1\text{-}b)$

$c_2 = (1\text{-}b) \cdot a$

$c_3 =$ T coefficient

$c_4 = 1 / (1\text{-}b)$

All additional equations were calculated by non linear regression and optimal parameter estimates obtained with the iterative non-linear least squares algorithm of Levenberg-Marquardt (SPSS 6 for Windows).

3.2.2. Composition of weight gain

To determine the composition of gilthead seabream of various sizes, fish were sampled along the growth cycle to obtain a wide range of increasing weights. Fish were used from the trials as used for growth prediction and feed intake. As the composition and energy content of the growing fish could be influenced by the nutritional status as well as composition of diet, estimation of body composition relied on data obtained with fish fed the same commercial diet throughout the observation period. Therefore by sampling the fish, attention was paid to use fish fed the commercial (Matmor) diet, and under similar feeding and growing conditions. Fish undergoing maturation were also not considered, as during that time body composition is not representative. Thirty-three groups of fish of approximately the same average size (fish differing by more than 10% from the mean were excluded) were sampled. To ensure sufficient sample material for all analyses, fish numbers for each group ranged from a minimum of 10 individuals to up to 100 depending on the fish size (Table A2, attachment). Fish of the same size were combined, frozen and stored for further analysis.

3.3 Results

3.3.1 Growth and feed intake

To establish a predictive model of the growth potential of gilthead seabream over the whole growing period, the growth and feed intake data collected from various trials (see Table A1, attachment) were described by a mathematical function. The relationship between weight gain (g) as the dependent and body weight (kg) as the independent variable was not linear and the data could be fitted best to a ln - ln function. The antilog of the function describes the allometric relationship common in biological measurements:

where BW = Body weight (in kg) of fish for sizes from 1 to 450g
T = Temperature between 20 - 26°C
n = sample size of 92

$$\text{Weight gain (g fish}^{-1}\text{ day}^{-1}) = 4.35\ (\pm 0.23) \cdot \text{BW (kg)}^{0.63\ (\pm 0.031)} \quad (1)$$

$r^2 = 0.87$

or including the temperature effect as an additional variable

$$\text{Weight gain (g fish}^{-1}\text{ day}^{-1}) = 0.92\ (\pm 0.31) \cdot \text{BW (kg)}^{0.613\ (\pm 0.029)} \cdot e^{0.065\ (\pm 0.014) \cdot T} \quad (2)$$

$r^2 = 0.89$

Transforming this equation accordingly, final weight BW_t can be predicted after t days starting from initial weight BW_0

$$BW_t = (BW_0^{\ 0.369} + 0.000356 \cdot e^{0.065 \cdot T} \cdot \text{days})^{2.710} \quad (3)$$

The feed intake caan be described in a similar manner

$$\text{Feed intake (g fish}^{-1}\text{ day}^{-1}) = 8.00\ (\pm 0.35) \cdot \text{BW (kg)}^{0.702\ (\pm 0.027)} \quad (4)$$

$r^2 = 0.93$

and including the effect of water temperature

$$\text{Feed intake (g fish}^{-1}\text{ day}^{-1}) = 1.38\ (\pm 0.39) \cdot \text{BW (kg)}^{0.681\ (\pm 0.026)} \cdot e^{0.073\ (\pm 0.012) \cdot T} \quad (5)$$

$r^2 = 0.94$

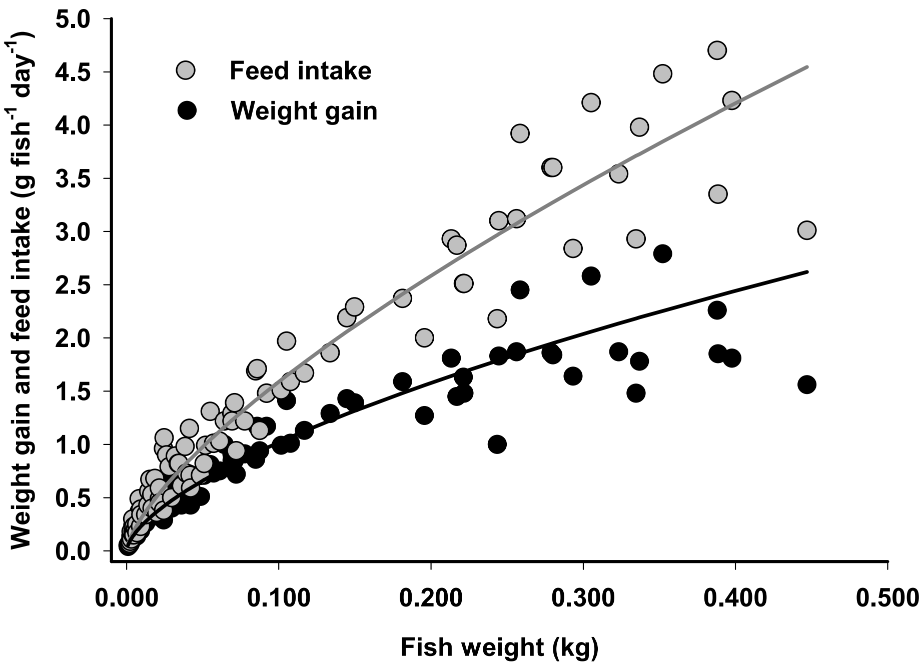

***Figure 1**: Daily weight gain (g) and feed intake (g) in relation to increasing body weight in gilthead seabream fed to satiation.*

Figure 1 depicts the relationship between daily weight gain (g), feed intake (g) and the body weight (kg) of fish. The lines describe the relationship at an average water temperature of 23°C for both feed intake and weight gain. As can be seen, absolute weight gain as well as the amount of food eaten increases with increasing weight, while feed intake increases at a higher rate than weight gain.

3.3.2 Composition of weight gain

The whole body composition of gilthead seabream from 1 to 420g is shown in Figure 2.

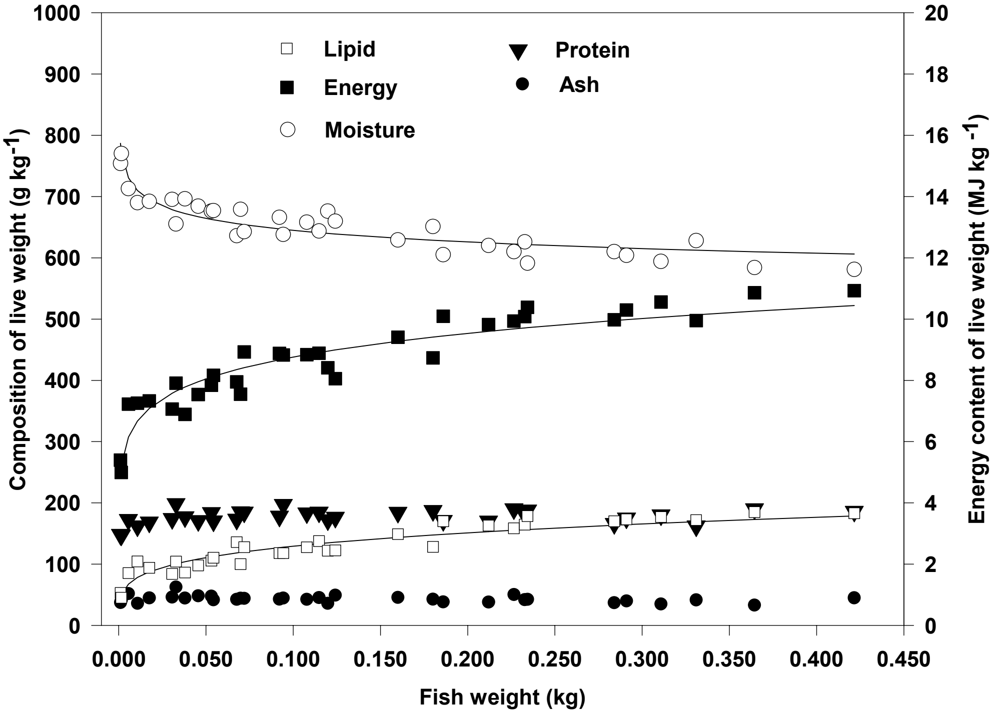

***Figure 2**: Proximate body composition (kg^{-1} WW) of gilthead seabream at various sizes and fed a standard diet to satiation (n=33, each data point represents analysis of a group of fish, corresponding equations (6) to (8) are presented in text).*

As can be seen protein and ash concentrations did not change with increased fish size and were, on average, 177 (± 11.5) and 43.3 (± 5.3) g kg^{-1} respectively. In contrast, moisture, lipid and energy concentrations showed deviations from linearity with increasing fish weight and could be best fitted to the allometric functions shown below (n = 33):

$$\text{Energy (MJ kg}^{-1}) = 11.6\ (\pm 0.3) \cdot \text{BW (kg)}^{0.122\ (\pm 0.009)} \quad r^2 = 0.88 \quad (6)$$

$$\text{Lipid (g kg}^{-1}) = 217\ (\pm 9.2) \cdot \text{BW (kg)}^{0.225\ (\pm 0.019)} \quad r^2 = 0.86 \quad (7)$$

$$\text{Moisture (g kg}^{-1}) = 584\ (\pm 6.0) \cdot \text{BW (kg)}^{-0.043\ (\pm 0.003)} \quad r^2 = 0.82 \quad (8)$$

The moisture (y) and lipid (x) concentrations (g kg^{-1}) were inversely related and could be described by the linear function:

$$y = 796\ (\pm 6.6) - 1.12\ (\pm 0.00)\ x \qquad r^2 = 0.94 \qquad (9)$$

3.4 Discussion

3.4.1 Weight gain and feed intake

In contrast to terrestrial animals fish seem to grow continuously, growth does not cease and reaches an asymptote, which in aquaculture however might never be attained. Growth rates in aquaculture have been in the past typically described using the specific growth rate (SGR) or as absolute growth in g per day. As growth is affected by temperature like in all poikilotherms, it increases with increase in temperature up to an optimum above which growth decreases, until the upper lethal temperature is reached. Although SGR and absolute weight gain are dependent upon feed intake and water temperature, their main dependence is on the size of the fish, and as a result they cannot be compared among groups of fish having different weights.

A model that fits the growth of the salmonids was developed by Iwama and Tautz (1981) and is based on cube root of weight:

$W_t^{0.3333} = W_0^{0.3333} + (T/1000) \cdot t$

where W_0 = initial weight (g)

W_t = weight (g) at time t

T = avg. temperature in °C

t = time in days

This growth model has been modified by Cho and Woodward (1989) and Cho (1990) by incorporating the thermal-unit growth coefficient (TGC) as follows:

$TGC = (W_t^{0.3333} - W_0^{0.3333}) / \Sigma\ (\text{temperature}(°C) \cdot \text{days})$

the expected live weight gain for rainbow trout could then be predicted after Cho (1992) as follows:

$W_t = [W_0^{0.3333} + \Sigma\ (0.00153 \cdot T \cdot \text{days})]^3$

This equation in its general form is equivalent to the formula for seabream of this study (equation 3), which of course is not too surprising as biological principles of fish growth should be the same. The growth potential itself, (and therefore the coefficients of the equation) would be of course typical for different fish species, and even different genetic strains.

3.4.2 Composition of weight gain

Because a large proportion of the energy and protein consumed by the fish is retained as growth, its carcass composition is a major factor determining the subsequent energy requirement of fish. When measuring whole body composition of fish at increasing sizes, each unit weight gain is assumed to equal the body composition at that size. Dry matter and fat content are generally the most variable factors in fish and can increase dramatically especially for the growth period of smaller fish (>100g) which is depicted in Figure 2. Therefore nutrient and energy gains should be measured at relatively short size intervals. In gilthead seabream, the protein content per kg live weight was found to range between 147 and 199 and be on average 177g. On the other hand, there is a strong inverse linear relationship between moisture and lipid (equation 9) in growing fish and consequently caloric content which ranges from 5 to close to 11 MJ kg^{-1} live weight. Lipid levels rise from 45 up to 183g kg^{-1} for the market sized seabream of about 400g. This is due to increase in weight only, as the fish were fed the same food. This has been documented in many fish (Love 1970; Shearer 1994), as well as in higher animals which deposit more fat and less moisture as they reach maturity. Phenotypic differences in composition might also exist between strains of trout (Ayles *et al*. 1979) but the seabream used in this study belonged to the same genetic group.

Table 1: *Whole body composition of some cultured fish species (kg^{-1} WW) in comparison to gilthead seabream.*

Fish species	Weight g $fish^{-1}$	DM g	Protein g	Lipid g	Energy MJ	References
Atlantic salmon	2500	?	187	194	12.0	Hillestad *et al*. 1998
Gilthead seabream	400	393	177	183	10.4	this study
European seabass	320	389	184	148	10.5	Lanari *et al*. 1999
Rainbow trout	300	346	172	146	9.7	Dias *et al*. 1999
Common carp	1200	300	154	120	8.3*	Zeitler *et al*. 1984
Tilapia	400	298	180	73	7.1	Viola *et al*. 1994
Red drum	390	289	170	69	6.7*	Thoman *et al*. 1999
Turbot	800	250	174	38	5.4	Regost *et al*. 2001

* Calculated using 23.6 and 39 kJ g^{-1} for protein and lipid respectively

Some examples of body composition of several fish species at approximate market size are given in Table 1. These results confirm, that protein concentrations are more conserved for a number of fish species, ranging from 170g to 187g kg^{-1} whereas lipid levels are more varied (Table 1). With a lipid content of up to 183g kg^{-1} live weight (Figure 2) one might add gilthead seabream to the category of a ‘fat’ fish, compared to some other cultured fish.

Therefore, in estimating requirements for tissue deposition and growth, wide variations between species, especially in terms of energy are expected based on the differing tissue composition. For example, relatively fat Atlantic salmon require more dietary energy per unit of live body weight than leaner fish such as red drum or turbot. Fish containing more moisture (less dry matter) require less energy for newly deposited growth.

4 Metabolic body weight

4.1 Introduction

Fish require energy for maintaining normal processes of life such as blood circulation, osmoregulation, excretion and movement, regardless of whether or not feed is consumed. Depending on the activity, several metabolic levels can be distinguished: standard, routine and active metabolism (Fry 1957; Brett 1962). Metabolic rate, at all levels of activity, depends largely on the size of the fish, and is proportional to the metabolic body weight in the form of $a \cdot BW(kg)^b$. Two major methods have been used to determine energy requirements in animals: direct and indirect calorimetry, however, most researchers have used indirect calorimetry in fish. The latter method estimates energy demand of fish indirectly through measurements of oxygen consumption, but can also include comparative carcass analysis.

The comparative slaughter technique was employed in this study to measure the caloric value of the tissues utilised during starvation. This method was chosen as the most feasible and applicable, fish could be kept in groups in a tank, move freely and the duration of each testing period was sufficiently long. The daily loss of energy as well as protein could be thus calculated.

4.2 Material and Methods

For determining the energy and protein loss following starvation, additionally half of the fish from some of those groups described above (23 of a total of 33 groups, Table A2 attachment) were stocked in outdoor 200L tanks for 16 to 40 days - also depending on size - without being fed. These starvation trials were undertaken during the whole year and the water temperature changed accordingly to the season (Table A3, attachment). After the starvation period, fish were sacrificed and stored at -20°C until further analyses. In calculating energy and protein loss on starvation the fish sampled initially were considered representative in body content with those kept for a further time period.

4.3 Results

From the comparative slaughter analysis the daily energy and protein loss during starvation was calculated for each weight group (Table A3, attachment). Daily energy and protein losses per fish plotted against the mean weights of the fish after starvation are shown in Figure 3.

The relationships between daily energy and protein loss and fish weight were not linear and results were fitted to ln - ln functions as have traditionally been used by animal nutritionists to express metabolic body weight (MBW). The antilog of these functions describes the allometric relationship common in biological measurements.

The daily loss of energy per fish can be described as follows:

Energy loss in kJ $fish^{-1}$ day^{-1} = $42.5\ (\pm 7.6) \cdot BW(kg)^{-0.83\ (\pm 0.09)}$ $r^2 = 0.97$ (10)

and the daily loss of protein per fish

Protein loss in g $fish^{-1}$ day^{-1} = $0.42\ (\pm 0.12) \cdot BW(kg)^{-0.70\ (\pm 0.13)}$ $r^2 = 0.90$ (11)

The expressions $kg^{0.83}$ and $kg^{0.70}$ can thus be described as the metabolic body weights for energy and protein respectively.

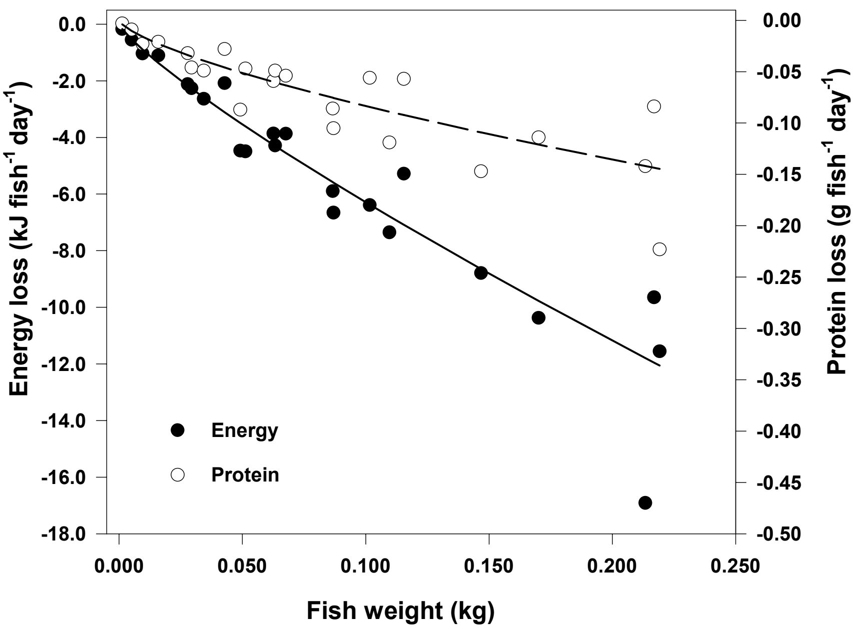

***Figure 3**: Energy (kJ day^{-1} $fish^{-1}$) and protein loss (g day^{-1} $fish^{-1}$) in gilthead seabream during starvation (at 23 ± 1.8 ℃, n = 23, each data point represents analysis of ten fish, fish weight is the geometric mean of initial and final weights after several days of starvation, corresponding equations (10) and (11) are presented in text).*

4.4 Discussion

Most researchers have used a logarithmic relationship to describe the metabolism - body weight relationship in higher animals as well as in fish. Glass (1969) found, that values derived by using iterative least square fitting were more accurate, however, the difference between the methods was small, and therefore the allometric function was used with gilthead seabream.

In this study starvation measurements were used to examine the relationship between energy or protein changes and body weight. The best fit of the function between energy and body weight changes occurred when body weight was raised to the power of 0.83 (equation 10). A considerable amount of literature exists on the estimation of the value for this exponent. Values for different fish species vary widely as reviewed by Hepher (1988) with 0.82 (n = 99) being the average value. Beck (1987) collected data of several starvation experiments in trout and found an average exponent of 0.833 (n = 63) for fish between 8 to 400g. Hogendoorn (1983) determined an exponent of 0.86 for African catfish and Cui and Liu (1990) found a common value of 0.855 in six different teleost species. Cho (1992) used 0.824 for rainbow trout. However, many others apply the approximation of 0.80 following the recommendation of Brett and Groves (1979). This means that the rate of increase in metabolism with fish weight is higher than that in birds and mammals.

Metabolic rate is a measure of the metabolic activity related to weight, and it decreases with increasing size at constant temperature. For poikilothermic fish, temperature has an important effect on their metabolism, although its importance may be species-specific. In this study metabolism was measured at ambient temperatures which ranged from 20 to 26°C and at this range the temperature effect was found to be negligible compared to the size effect. Similar findings were reported by Requena *et al*. (1997) who measured rates of oxygen consumption in gilthead seabream with increasing temperatures from 20 to 28°C. After a few days of acclimatisation, the fish did not show a significant increase in metabolic expenditure, even at the higher temperatures.

The energy loss during starvation (E_{starv}) for gilthead seabream was 42.5 kJ BW(kg)$^{-0.83}$ day^{-1} (equation 10). The average temperature during these measurements was close to 23°C. Considering the different temperatures, this value is quite close to the fasting heat production of 42 kJ BW(kg)$^{-0.82}$ in trout at 20°C (Cho and Kaushik 1990). Recalculation of Beck's data (1987) gave a value of 49 kJ BW(kg)$^{-0.833}$ day^{-1} at 20°C for trout and Meyer-Burgdorff *et al.* (1989) observed a low energy loss of 25 kJ BW(kg)$^{-0.80}$ day^{-1} in tilapia whereas Hepher *et al.* (1983) determined an energy loss in red tilapia of 40 kJ BW(kg)$^{-0.80}$ day^{-1}.

Similar to the energy metabolism the best fit between protein loss and body weight in gilthead seabream was reached using a metabolic body weight calculated with an exponent of 0.70 (equation 11). For comparison, using an exponent of 0.83 explained 13% less of the variance. Data correlating protein loss to different fish weights are sparse and most authors have assumed a common exponent for the relationship of energy loss and protein loss to body weight. However, an exponent of 0.739 (N = 45) was calculated for trout of 5 to 400g from various data in the literature (Beck 1987). This value is close to the exponent of 0.70 found in this study and it also reflects the difference in the relationships between energy and protein and increasing body weight (Figure 3), where percent protein is generally conserved in regard to fish size. It is thus clear that protein and energy loss cannot be described by the same metabolic body weight.

The protein loss (CP_{starv}) of gilthead seabream was 0.42g BW $(kg)^{-0.70}$ day^{-1} according to equation (11) and this value is comparable to those reported in other fish species. Estimates of protein losses for several fish species averaged 0.32g $BW(kg)^{-0.75}$ day^{-1} (Bowen 1987) whereas in trout losses were 0.53g protein $BW(kg)^{-0.739}$ day^{-1} (Beck 1987). Pfeffer *et al.* (1977) found obligatory protein losses in carp to be between 0.28-0.55g $BW(kg)^{-1}$ day^{-1} and Meyer-Burgdorff *et al.* (1989) reported 0.38g protein $BW(kg)^{-0.80}$ day^{-1} lost in tilapia. Losses of protein for maintenance consists mainly of losses from the integument and intestine, from oxidation and conversion of amino acids and from protein turnover. Losses from these causes are considered not likely to differ appreciably between species (Cowey 1994).

Some criticism has been put forward using starvation as a means to determine maintenance energy requirements for a longer period of time since the rate of loss of body tissues is higher during first weeks of fasting compared to the subsequent weeks (Hepher *et al*. 1983). However this would only influence the coefficient a in the expression a $(kg)^b$ and moreover, loss at starvation is only an approximation of maintenance energy requirements.

Taking this into account, it is still quite striking how close the values really are when comparing the different values of E_{starv} and CP_{starv} obtained for the various fish species, possibly since only cases were considered where losses due to starvation were measured. On the other hand the difference to homoeothermic vertebrates is remarkable, as their energy requirements for basal metabolism are up to 10 fold higher averaging 300 kJ $BW(kg)^{-0.75}$ day^{-1} (Kleiber 1965). The low value for fish can be explained by lack of expenditure for thermoregulation, the lack of gravity and the mode of nitrogen excretion (ammoniotelism).

5 Digestibility of energy and organic compounds

5.1 Introduction

Food is the principal operating cost in the production of fish and for aquatic feeds the main protein and energy source has traditionally been fish meal. However, there are limits to the continued expansion of aquaculture based upon feeds using fish meal and fish oils, which are costly and limited in supply. Recently, farming fish using wild fishery resources has led to some criticisms (Naylor *et al*. 1998; Pauly *et al*. 2002) and research has concentrated on replacing fish meal with cheaper ingredients of either animal or vegetable origin (Kaushik 1990; Higgs *et al*. 1995). The production of successful fish feed formulae relying less on fish meal requires therefore accurate information on the nutritive value of more economical protein sources. Knowledge of the digestibility of these various alternative ingredients is a basic tool for formulating diets and is required to indicate the availability of energy and nutrients (Cho and Kaushik 1990). Ideally the nutrient requirements of fish and the nutrient concentration of a foodstuff should be expressed in units of availability so that least-cost formulations can optimise the balance between nutrient requirements and the cost of feeds.

Digestibility studies, using a range of feed ingredients, have been carried out in various fish species including rainbow trout (Cho and Slinger 1979), Atlantic cod (Lied *et al*. 1982), channel catfish (Wilson and Poe 1985) and tilapia (Hanley 1987; Anderson *et al*. 1991). Dependency of digestibility on feeding levels (Henken *et al*. 1985) and temperature has also been studied (Kaushik 1981; Choubert *et al*. 1982). Carbohydrate digestibility has been examined in Atlantic salmon by Arnesen and Krogdahl (1993), and these workers determined the maximum tolerable level of carbohydrate in diets for this species. The digestibility of carbohydrate in rainbow trout has been shown to depend on the level of inclusion (Rychly and Spannhof 1979; Henrichfreise and Pfeffer 1992).

Few digestibility studies have been reported for gilthead seabream, a species grown in aquaculture operations in the Mediterranean region (Vergara and Jauncey 1993; Nengas *et al*. 1995; Fernández *et al*. 1998). Most nutritional research has concentrated on salmonids, cyprinids, ictalurids and tilapias, and differences between species are expected. Therefore, for most efficient feed formulation, nutrient availability should be determined for each species.

The nutritive value of compound diets will depend on the digestibility of the individual ingredients, but potential interactions among ingredients should also be considered and the additivity of individual digestibility demonstrated. Ideally one should be able to express the nutritional value of a diet on the basis of its digestible energy (DE) and digestible crude protein (DCP). As some ingredients cannot be fed as sole feed, knowledge of digestibility of single feeds must be based on evaluation of digestibility studies in which test ingredients have been blended with reference diets of known digestibility. By using a reference ingredient care must be taken that the inclusion level of the nutrient in question is high enough to make the interpretation reproducible.

The objectives were:

1) To provide specific information required for the formulation of diets on the basis of DE and DCP used for further trials with seabream.
2) To determine digestibility values for gross energy, crude protein, lipid and crude carbohydrates of various local available ingredients which might be used for seabream nutrition.
3) To ascertain whether digestibility values of compound diets can be accurately predicted from the digestibility of the constituent ingredients.

5.2 Material and Methods

Much consideration was given to the choice of marker and ways of collection of faeces. Chromic oxide (Cr_2O_3) has been used repeatedly in digestibility studies, among others like acid insoluble ash, titanium oxide and yttrium. Chromic oxide was chosen as it proved to be very sensitive and gave reproducible results with the equipment that was available at NCM. Preliminary tests were performed to test the various options of faecal collection, mainly stripping and collection or siphoning. As the faecal matter of gilthead seabream is dissolving rapidly in the water (personal observation) the option of stripping was chosen.

As faeces collected by this method may be contaminated by mucus, urine or sexual products the following procedures were followed:

- Experiments were conducted during May to October as gilthead seabream spawns from December through March.
- Fish were anaesthetised with ethylene-monoglycol-ether prior to handling
- The bladder was emptied by slight pressure and the area around the anus dried with a towel
- Faecal matter was collected by exerting gentle pressure on the area from the anal fin to the anus.

Feed ingredients selected for determining digestibility were chosen according to availability and potential source of animal feeds in Israel (Table 2).

Digestibility measurements were carried out in successive trials: first on single feed ingredients using a range of relatively high protein components of vegetable and animal origin (Table 3). As some ingredients cannot be fed as sole feed, they were blended with a reference substance of known digestibility. The reference ingredient of choice was fish meal, due to its availability, palatability and ease of pelleting. In the course of the trials as well as the additional feeding trials, four different sources of fish meal (A through D) were used and the digestibility was tested for each batch.

The second step was testing feed ingredients traditionally used as energy sources like carbohydrate rich diets. Additionally, the carbohydrate sources were combined with the fishmeal at two dietary proportions to test the effect of inclusion level of carbohydrate on digestibility (Table 4). This was another reason to choose fish meal as a reference diet, since it does not contain significant levels of carbohydrates.

In the third step, five diets were formulated to combine some of the single ingredients tested before. This was done to test the additivity of the digestibility coefficients mainly for energy and protein. The compound diets were formulated to contain protein and energy levels considered to be practical for the culture of gilthead seabream (Table 5).

All the diets were prepared as previously described.

5.2.1 Feeds and feed formulations

Table 2 shows the nutrient composition of all the ingredients used in the digestibility trials and growth trials, as obtained by the analytical methods described in chapter 2.4.

__Table 2__: Nutrient composition of the feed ingredients (kg^{-1} DM) used in digestibility and growth trials of gilthead seabream.

	GE MJ	CP g	CL g	CC g	Ash g
Fish meal A[1] (FM A)	21.70	763	102	-	135
Fish meal B[1] (FM B)	21.48	636	108	-	130
Fish meal C[1] (FM C)	22.60	700	135	-	125
Fish meal D[1] (FM D)	21.62	681	151	-	151
Meat meal[2] (MM)	20.90	640	127	-	173
Poultry meal[3] (PM)	21.80	674	154	-	156
Squid meal[4] (SQM)	23.70	753	102	-	87
Blood meal[5] (BM)	24.00	897	26	-	47
Fish oil (FO)	38.50	-	1000	-	-
Soybean meal[6] (SBM)	19.70	484	22	432	62
Corn meal (CM)	19.16	123	42	818	17
Corn starch (CS)	17.46	-	-	989	11
Wheat meal (WM)	19.00	136	22	824	18

(-) not determined

[1] Fish meals A, B, C and D, different sources from Argentina, Denmark, Iceland

[2] Meat meal, local

[3] Poultry by-product meal, local

[4] Squid meal, sun-dried, India

[5] Blood meal, spray-dried

[6] Soybean meal, solvent extracted

***Table 3**: Formulation and nutrient composition of diets used for determination of digestibility of protein rich ingredients.*

Diets	MM979	PM979	SQM979	FM979 (A)	FM979 (B)	FM979 (C)	FM879 (C)	BM422	SBM250	SBM729	SBM912
Component (g kg $^{-1}$)											
Fish meal (A)				979							
Fish meal (B)					979						
Fish meal (C)						979	879	557	729	250	
Meat meal	979										
Poultry meal		979									
Squid meal			979								
Blood meal								422			
Soybean meal									250	729	912
Fish oil							100				67
Vitamin mix	14	14	14	14	14	14	14	14	14	14	14
Cr_2O_3	7	7	7	7	7	7	7	7	7	7	7
Analysis, kg $^{-1}$ DM											
Gross energy, MJ	20.40	21.65	23.56	21.72	21.07	22.35	22.96	22.60	20.90	20.10	21.0
Crude protein, g	626	670	744	740	618	685	612	766	635	530	434
Crude lipid, g	122	152	99	95	87	135	207	79	106	56	72
Crude carbohydrate, g	-	-	-	-	-	-	-	-	132	319	422
Ash, g	174	157	85	139	142	131	126	107	127	95	72
Chromium, g	5.22	5.34	5.68	5.80	6.02	5.47	5.20	5.54	5.34	5.75	5.1

Table 4: *Formulation and nutrient composition of diets used for determination of digestibility of carbohydrate rich ingredients at two dietary inclusion levels.*

	FM (D) 982	SBM 250	SBM 732	CM 200	CM 400	CS 200	CS 400	WM 200	WM 400
Component (g kg $^{-1}$)									
Fish meal (D)	982	732	250	782	582	782	582	782	582
Soybean meal		250	732						
Corn meal				200	400				
Cornstarch						200	400		
Wheat meal								200	400
Vitamin mix	10	10	10	10	10	10	10	10	10
Cr_2O_3	8	8	8	8	8	8	8	8	8
Analysis (kg $^{-1}$ DM)									
Gross energy, MJ	21.06	21.41	20.23	20.96	19.83	20.89	20.02	20.23	20.13
Crude protein, g	687	628	534	577	452	558	435	580	481
Crude lipid, g	129	112	56	121	95	110	88	118	92
Crude carbohydrate, g	-	116	307	163	343	193	369	163	313
Ash, g	159	144	103	139	110	139	109	139	114
Chromium, g	6.23	6.09	5.86	6.09	6.30	6.30	6.43	6.48	6.19

Table 5: *Formulation and proximate analysis of five compound diets used to determine additivity of digestibility.*

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Component (g kg $^{-1}$)					
Fish meal (C)	350	350	320	300	140
Meat meal	200		200	180	130
Poultry meal			150		140
Blood meal		150		160	135
Soybean meal	220	220			160
Wheat flour	150	150	250	250	190
Fish oil	50	80	50	70	70
Vitamin mix	14	14	14	14	14
Mineral mix	8	28	8	18	13
Cr_2O_3	8	8	8	8	8
Analysis (kg $^{-1}$ DM)					
Gross energy, MJ	21.72	21.89	21.63	21.38	21.20
Crude protein, g	511	521	508	515	516
Crude lipid, g	138	138	146	139	149
Crude carbohydrate, g	236	217	231	229	220
Ash, g	115	124	115	117	115
Chromium, g	5.82	6.25	6.44	6.03	6.23

5.2.3 Experimental fish and faeces collection

Seabream (300 - 450g) were stocked (in groups of about 20- 25) in eight outdoor 400L tanks supplied with flow-through sea water at ambient temperature from May to November (23-26°C). Two or three replicate groups of fish were adapted to each experimental diet for 4 days prior to the start of faecal collection. Fish were fed once a day to satiation, which is at that size approximately 1% of their biomass. Feeding was done late in the evening at sunset and the next day early in the morning the faeces were collected, pre-trials showed, that most efficient faecal collection could be achieved in this way. Prior to the actual faecal sampling, 4 - 5 fish were taken out of the 400L tanks, moved to a smaller holding device containing the anaesthetic. When fish showed signs of quieting down, each fish was taken out by hand, dried with a soft cloth and faeces collected by lightly stripping along the anus. If fish did not release faeces easily, or there were signs that defecation had occurred already, the fish was released to the main tank. Sampling of faeces was performed up to six times for each dietary treatment with intervals of two days between sampling and faeces from the same tank collected over this period were pooled. In a few instances where the amount of faeces collected over the stripping period was too small, samples from replicate tanks of the same treatment were combined. No major mortalities or health problems were encountered during the experimental period and fish regained their appetite shortly after handling. Fish were feeding in the evening after faecal collection in the morning, which was taken as a positive sign of stress-free handling.

5.2.4 Chemical analysis

The combined faecal samples were oven dried at 60°C, ground with mortar and pestle and kept at 4°C for subsequent analyses. Feed samples were finely ground in a hammer mill using a 1 mm screen. Chromium (Cr) was measured by wet digestion after a modification of the analysis of Furukawa and Tsukahara (1966). Food and faeces containing chromic oxide Cr_2O_3 were digested in a mixture of perchloric acid, concentrated sulphuric acid and Na-molybdate in Kjeldahl digestion flasks at a temperature of 250°C. The resulting dichromate was determined in a spectrophotometer at 360 nm against Cr_2O_7 standard solutions.

5.2.5 Calculations

Apparent digestibility coefficients (ADC) were calculated according to the generally accepted equation (NRC 1993):

$$\text{ADC (\%)} = 100 - 100 \cdot (\%\, Cr_{Food} / \%\, Cr_{Faeces}) \cdot (\%\, Nutrient_{Faeces} / \%\, Nutrient_{Food}) \qquad (12)$$

The partial digestibility coefficients were calculated according to Schürch (1969):

$$DC_T = [DC_D - DC_R \cdot (1 - t)] / t \qquad (13)$$

where

DC_D = Digestibility coefficient of the nutrient in total diet (%)

DC_R = Digestibility coefficient of the nutrient in reference ingredient (%)

DC_T = Digestibility coefficient of the nutrient in test ingredient (%)

t = Contribution of nutrient of test ingredient to total diet (%)

t =

100 - [(nutrient concentration in R · inclusion of R in D %) / (nutrient concentration in D)]

where R = reference ingredient

T = test ingredient

D = R + T, whole diet

5.2.6 Definitions

Index of Similarity: a value that describes the similarity between the predicted and the measured digestibility parameters of a nutrient. A value of 100 means that the two estimates used in the comparison are identical.

Index of Similarity = predicted digestibility / measured digestibility · 100

5.3 Results

The apparent digestibility values for crude protein, lipid, carbohydrate and energy for the individual ingredients that could be fed alone were calculated using equation (12) and are included in Table 6. The results of the partial digestibilities of the single test ingredients using equation (13) are presented in Table 7.

Table 6*: Apparent digestibility coefficient (%) of energy and proximate nutrients of protein high ingredients (mean ± SD where appropriate).*

	GE	CP	CL
Fish meal (A)	89[2]	88[2]	96[2]
	89-89	88-89	95-97
Fish meal (B)	88[2]	88[2]	95[2]
	87-88	88-88	95-95
Fish meal (C)	80[3]	83[3]	95[3]
	± 0.63	±1.73	± 1.43
Meat meal	78[2]	79[2]	88[2]
	78-78	78-79	87-89
Poultry meal	79[2]	80[2]	93[2]
	78-80	79-80	92-95
Squid meal	85[1]	88[3]	83[3]
		± 1.73	± 5.03

[1 -3] number of replicates

***Table** 7: Apparent digestibility (%) of energy and nutrients of whole diets (reference + test ingredient) and calculated ADCs of the individual high protein ingredients.*

	GE	CP	CL	CC
Whole diets				
BM422 + FM (C)	81[1]	86[3] ± 0.5	-	-
FO + FM (C)	83[2]	83[2]	95[2]	-
	82-84	83-83	95-96	
SBM250 + FM (C)	78[1]	84[1]	93[1]	-7[1]
SBM729 + FM (C)	70[1]	85[1]	94[1]	9[1]
SBM912 + Fish oil	69[1]	87[1]	95[1]	25[1]
Single ingredients				
Blood meal	83[1]	90[3] ±1.3	-	-
Fish oil	96[2]		95[2]	
	90-102		94-96	
Soybean meal [a]	71[1]	88[1]		-7[1]
Soybean meal [b]	66[1]	86[1]	-	9[1]
Soybean meal [c]	65[1]	87[1]		25[1]

[1 - 3] number of replicate treatments,

[a - c] increasing inclusion levels of test ingredient

Table 8*: Apparent digestibility (%) of energy and nutrients of whole diets (reference + test ingredient) containing two levels of carbohydrate rich feed ingredients (mean of two replicates)*

Diets	GE	CP	CL	CC
Fish meal (D)*	77	80	90	
	75-78	79-81	90-91	
SBM250 + FM	74	83	88	-5
	74-74	82-83	88-88	2- (-12)
SBM732 + FM	69	85	74	14
	67-71	85-85	73-74	9-19
CM200 + FM	77	79	92	21
	76-77	78-80	92-92	19-23
CM400 + FM	67	77	90	44
	64-70	76-78	89-90	36-51
CS200 + FM	79	80	94	64
	79-80	79-80	93-94	64-64
CS400 + FM	78	78	93	72
	78-78	77-78	91-94	70-73
WM200 + FM	74	78	91	39
	74-75	78-79	91-92	36-42
WM400 + FM	72	79	90	51
	70-75	79-80	90-91	44-57

* reference ingredient for the test ingredients under study

Table 9: *Apparent digestibility (%) of energy and nutrients of carbohydrate rich ingredients that had been fed with reference ingredient (mean of two replicates).*

Ingredient	GE	CP	CL	CC
Fish meal (D)	77	80	90	
	75-79	79-81	90-91	
Soybean meal [a]	65	92	40	-5
	65-65	89-94	38-42	2-(-12)
Soybean meal [b]	66	87	35	14
	64-68	87-88	32-37	9-19
Corn meal [a]	75	60	117	21
	74-77	44-75	116-119	19-23
Corn meal [b]	52	54	87	44
	44-60	44-64	83-91	36-51
Corn starch [a]	93	-	-	64
	91-95			64-64
Corn starch [b]	80	-	-	72
	79-81			70-73
Wheat meal [a]	62	43	119	39
	59-65	35-51	110-129	36-42
Wheat meal [b]	65	76	92	51
	59-71	71-81	83-102	44-57

[a - b] increasing inclusion levels of test ingredient in whole diet

The crude protein digestibilities were high on average and ranged from a minimum of 79% for meat meal to a maximum of 90% for blood meal. Special attention should be drawn to the difference in digestibility coefficients among the four fish meals A through D. Digestibility was low for fish meal D at 80% and 77% for protein and energy respectively compared to a digestibility of 88% and 89% for protein and energy of fish meal A. Digestibility of soybean meal protein regardless of the inclusion level and combination with fish meal or fish oil was in all cases close to 87% (Tables 7 and 10). Lipid digestibilities on average were also high, 95-96% for fish oils, whether incorporated as part of the fish meal itself or as pure fish oil (Tables 6, 7). Greater differences were seen in carbohydrate digestibility of various sources, the least digestible sources were corn meal and soybean meal, and the best cornstarch and wheat meal (Table 10). Some differences could be seen at the higher inclusion levels of carbohydrate in the diet, which decreases the digestibility, but the effect was not seen in wheat meal. Energy digestibility reflects the sum of the digestibility of all the energy yielding nutrients.

To sum up the values obtained in the digestibility studies and used later on in the feeding trials, Table 10 specifies the measured amounts of DE and DCP of each ingredient used.

***Table 10**: Concentrations of digestible energy and nutrients in practical feed ingredients as determined in digestibility trials (per kg DM).*

	DE (MJ)	DCP (g)	DCL (g)	DCC (g)
Fish meal (A)	19.3	671	98	
Fish meal (B)	18.9	560	103	
Fish meal (C)	18.1	581	128	
Fish meal (D)	16.7	545	136	
Meat meal	16.3	506	112	
Poultry meal	17.2	539	143	
Squid meal	20.1	663	85	
Blood meal	19.9	807	(16)	
Fish oil	36.9	-	950	
Soybean meal	13.0	421	(8)	108
Corn meal	9.9	95	(37)	360
Corn starch	14.0	-	-	712
Wheat meal	12.4	103	(20)	420

One of the objectives of the digestibility trial was, to test the additivity of digestibility values for energy, protein, lipid and carbohydrates for single ingredients. Table 11 shows the apparent digestibility values for the compound diets formulated as in Table 5. The measured digestibility of the various nutrients was as follows: crude protein ranged from 80 to 88%, lipid from 88 to 95%, carbohydrate from 24 to 78% and energy from 71 to 86%.

To test if the digestibility coefficients for energy and the nutrients are additive, the results of the five diets combining the various single ingredients, as measured in situ in the fish digestibility trials and as predicted from the sum of digestibility values from Table 10 were compared. The predicted digestibility values for the nutrients (protein, lipid, carbohydrate and energy) in the test diets were calculated on the basis of those of the individual ingredients (Table 10) and these were compared to the measured values using the similarity index (Figure 4). The index of similarity ranged from 95 to 104 (mean ± CV, 99.3 ± 3.3%) for crude protein, from 98 to 104 (100.9 ± 2.4%) for lipid, from 65 to 163 (103 ± 37%) for crude carbohydrate and from 92 to 109 (99.8 ± 6.2%) for overall energy (Figure 4).

***Table 11**: Comparison of measured and predicted apparent digestibility coefficients of five formulated diets.*

	Diet 1		Diet 2		Diet 3		Diet 4		Diet 5	
	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted
GE (MJ kg^{-1})	21.72	21.20	21.89	21.77	21.63	21.36	21.38	21.83	21.20	21.63
DE (MJ kg^{-1})	16.79	16.15	16.72	16.99	17.13	16.56	18.37	17.22	15.12	16.76
ADC (%)	77.3	76.2	76.4	78.0	79.2	77.6	85.9	78.9	71.3	77.5
CP (g kg^{-1})	511	500	521	506	508	487	515	503	516	500
DCP (g kg^{-1})	430	413	451	432	410	394	452	420	414	418
ADC (%)	84.1	82.5	86.6	85.4	80.7	80.8	87.7	83.6	80.2	83.7
CL (g kg^{-1})	138	131	138	139	146	147	139	143	149	138
DCL (g kg^{-1})	123	119	122	128	136	137	132	133	136	126
ADC (%)	88.8	91.3	88.4	91.9	93.4	93.3	94.8	92.7	91.4	91.4
CC (g kg^{-1})	236	219	217	219	231	206	229	206	220	226
DCC (g kg^{-1})	102	87	53	87	146	105	179	105	84	97
ADC (%)	43.2	39.7	24.3	39.7	63.4	51.0	78.3	51.0	38.1	43.0

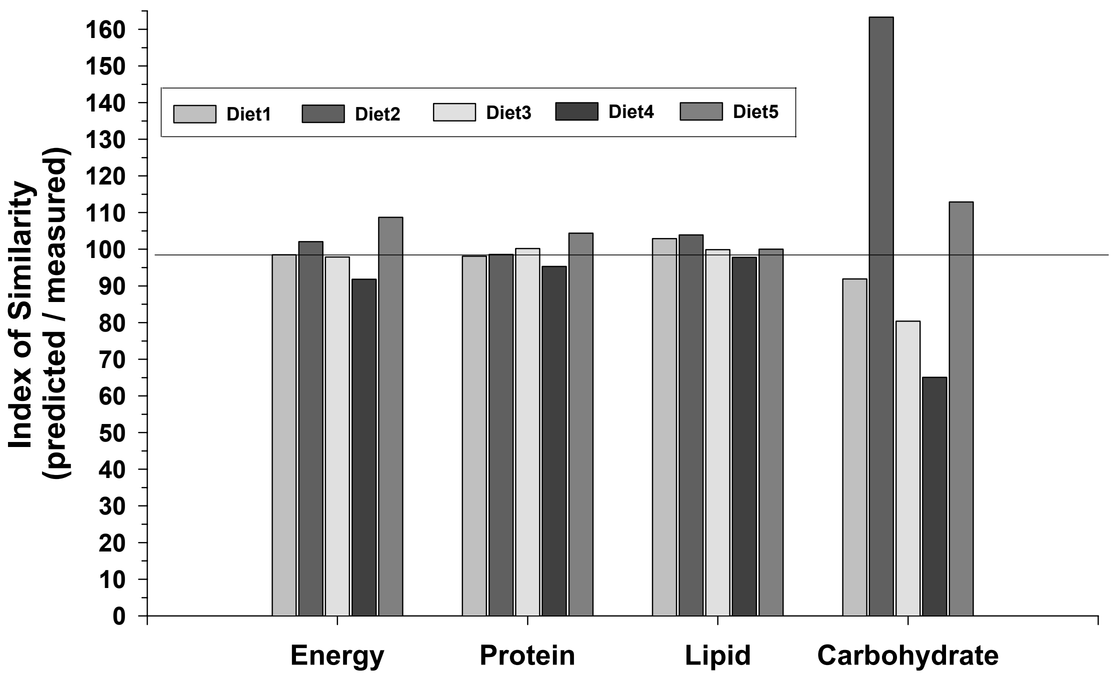

***Figure 4**. Index of similarity of predicted to measured ADCs (%) of energy, protein, lipid and carbohydrates in five compound diets.*

5.4 Discussion

Apparent digestibility measurements provide a good indication of the availability of dietary energy and nutrients, providing a basis upon which complete diets can be formulated. However, digestibility determinations are problematic in fish, mainly due to leaching from the faeces into the water before collection. Different methods for faecal sampling have been tested by several authors, among them: Austreng 1978; Choubert *et al*. 1982; Spyridakis *et al*. 1989; Cho and Kaushik 1990; Allan *et al*. 1999. Techniques included samples obtained by dissection, stripping, siphoning after the faeces are voided and settlement columns. One common feature for all those techniques is that ADC values calculated tended to increase in the following order: dissection along several regions of the intestine, stripping and sampling of voided faeces. In this work stripping was chosen for gilthead seabream since it yielded the most reliable results. This was confirmed recently by Fernández *et al*. (1996) who tested the above mentioned methods for faecal collection and concluded, that stripping was the best method for this species.

Apparent digestibilities of crude protein were high regardless of the origin of the protein. There was no correlation between the crude protein content of the diets and its digestibility. Protein from extracted soybean meal had an ADC of 87%, whether fed alone or in combination with fish meal (Tables 7 and 9). This is similar to the ADCs of 86.7% found for rainbow trout (Alexis *et al.* 1988) and 85% for channel catfish (Brown and Strange 1985). A slightly higher ADC value of 91% has been reported in another study with gilthead seabream (Nengas *et al*. 1995), but faecal collection was done by sedimentation.

There seem to be more variation in protein digestibility of animal meal and by-products than from plant proteins possibly due to processing procedures. Reported digestibility values of fish meals and animal by-products vary considerably depending on the source and treatment of the meal. Some of the differences arise due to different processing treatments such as heating, drum drying or spray drying. In this course of the present study four different fish meals A to D were tested, the treatment of those meals is unknown but the digestibility ranged from 80 to 88%. Digestibility of fish meal may be improved by employing low temperature in the drying process (Pike *et al*. 1990). Heat damage has also been shown to have a very significant effect on digestibility of protein of blood meal for fish. For example, digestibility of 40% were found by Vens-Capell (1983), 55% by Cho and Kaushik (1990) and 97% by Åsgård and Austreng (1986) when drum dried, flame dried and chilled ensiled blood meal were fed to rainbow trout. In a more recent study with trout Bureau *et al*. (1999) found also consistently high apparent digestibility coefficients of 96 - 99% for spray-dried blood products. In this study protein digestibility was found to be 90% for the spray dried blood meal fed to gilthead seabream.

Use of the protein digestibility obtained for individual ingredients (Table 10) to predict the protein digestibility of compound diets (Table 11) gave values very close to those measured (Figure 4). Therefore, it appears, that protein digestibilities can be considered to be additive.

Lipid digestibilities were generally high around 95% (Table 10), but it should be noted that only animal fats were tested in this study. The biggest differences in lipid digestibility were found between fish oil at 95% and squid meal at 83%. Takeuchi *et al.* (1979) showed, that the digestibility of lipids depends on their fatty acid composition and saturation level. Austreng *et al.* (1980) found a strong influence of lipid fatty acid composition, and thus the melting point of the fat, on lipid digestibility in rainbow trout. Squid oil is typically high in polyunsaturated fatty acids but the sun-drying process of the meal used in our study might have caused some oxidation. Oxidation of oils diminished their availability to red seabream (Sakaguchi and Hamaguchi 1979). The ADC of 95% for fish meal lipid is consistent with the value of 97% obtained for rainbow trout by Cho and Slinger (1979). Their value for digestibility of poultry by-product lipid was only 83% compared to the ADC of 93% obtained for gilthead seabream in this study (Table 6).

The highest variation in ADC's was found among the carbohydrate rich ingredients. Apparent crude carbohydrate digestibility varied considerably depending on source and slightly less due to inclusion level. ADC of carbohydrate in soybean meal was only 25% at the highest inclusion level (Table 7), whereas that of wheat was 51% (Table 9). Similar tendencies were found in trout, where native starches from different sources had digestibility ranging from 5% for potato to 60% for wheat (Bergot 1993), but the inclusion level of carbohydrate was also found to influence digestibility (Bergot and Breque 1983; Hemre *et al*. 1989; Pfeffer *et al*. 1995) as might processing (Vens-Capell 1984; Hemre *et al*. 1990; Pfeffer *et al*. 1991).

Therefore, when attempts were made to predict digestible carbohydrates in the five compound diets used (Figure 4), the range in the index of similarity was higher than for the other nutrient groups (65 - 163). The difference in the predicted as compared to the measured digestibility of carbohydrates in this study was not due to inclusion levels, as there was no significant difference for wheat, nor to different processing methods, as those diets were all prepared the same way. In addition it should be noted that carbohydrates were calculated by difference. Crude carbohydrates are a complex mixture of oligosaccarides as well as soluble and insoluble fibres. Clearly the components of the “carbohydrate” fraction must be better defined in order to better understand which fractions would influence digestibility.

Energy digestibility of the single ingredients reflected the sum of all the energy yielding nutrients as assumed. Energy digestibility of the single ingredients fed to gilthead seabream varied from 52% for corn meal to 89% for one of the fish meals. The use of the predicted values for energy digestibility in compound diets very close to the measured ones (Table 11, Figure 4).

In summary, the results of the digestibility trials provide evidence that the digestibility of energy, protein and lipids are additive in gilthead seabream and values determined for individual ingredients may be used for estimating nutrient digestibility in formulated compound feeds.

6 Efficiency of energy and protein utilisation

6.1 Introduction

In order to quantify the energy and protein requirement according to the factorial model an additional parameter, the efficiency of dietary energy and protein to deposit new body tissue as growth has to be examined. Energy and protein requirements are very complex as they are closely linked. Without protein there is no growth, but neither is there growth without energy. Since protein can function as an energy source in addition to its essential role for growth, the efficiency for growth is dependent on the balance between the supply of dietary non-protein energy and protein.

Compared to terrestrial domesticated animals, fish are commonly fed a higher protein diet (350 - 450g CP kg^{-1}) and are thought to preferably use protein as an energy source. As protein is an expensive ingredient and the metabolic end-products might negatively influence water quality parameters, the concept of protein sparing has been examined extensively in fish.

Several authors have described optimal dietary protein to energy ratios in rainbow trout (Kim and Kaushik 1992; Lanari *et al*. 1995), African catfish (Henken *et al*. 1986), yellowtail (Takeda *et al*. 1975; Shimeno *et al*. 1985), tilapia (Winfree and Stickney 1981; Shiau and Huang 1990), Atlantic salmon (Hillestad and Johnson 1994), common carp (Watanabe *et al*. 1987), European seabass (Tibaldi *et al*. 1991) and gilthead seabream (Kissil *et al*. 1982; Vergara *et al*. 1996b). However, despite this body of information, data concerning the optimal protein requirement for the same species are often controversial, due to discrepancies between results reported by various authors.

One of the factors affecting the dietary protein to energy ratios might be the use of fish of different weights, as protein requirements decrease with increasing fish size (Page and Andrews 1973; Kaushik and Luquet 1984; Masser *et al*. 1991). Another source of variation is the difference in digestibility coefficients of dietary energy and protein, which have not been defined clearly in many feeds. Furthermore, the methods of calculating the DE content may vary among authors, making it difficult to draw conclusions with regard to the optimal DCP/DE ratios. A further difference between the studies is the choice of feeding rate which could vary from specific percentage of biomass to *ad libitum* feeding. For instance, applying a daily feeding rate of 80g per kg biomass could have masked the effect of reduced feed intake in red drum fed high lipid diets (Williams and Robinson 1988). Some experiments were performed with purified diets, where feed intake was below the normal range, probably due to palatability problems, and rapid growth was not found (Sabaut and Luquet 1973). Since the growth rate is the criterion for which the protein and energy requirements are being established, it is reasonable that high growth rates should be achieved with high feed intake.

Therefore to determine the energy and protein efficiency in gilthead seabream the objectives were as follows:

a) Energy and protein utilisation at increasing feeding levels
b) Energy and protein utilisation at differing DCP/DE ratios
c) The optimal efficiency of protein utilisation

6.2. Energy and protein utilisation for maintenance and growth

6.2.1 Materials and Methods

Two growth trials A and B were performed with two groups of fish weighing 30g and 92g initially as shown in Table 12. Two different diets were used. Diet A was based mainly on fish meal and fish oil (Table 13) and steam pelleted to 2.4 mm diameter using the laboratory pellet mill. Diet B was a commercial diet, which has been used locally for seabream in the past (Matmor Inc.) and was supplied as 4 mm pellets. Formulation of Diet B according to the manufacturer is shown in Table 13.

Table 12*. Experimental set-up for growth trials A and B to evaluate effect of increasing energy and protein intake.*

	Trial A	Trial B
Fish	20 per tank	18 per tank
Initial weight	30.1g	92.5 g
Feeding level	starvation, low, medium and high feeding level	
Feeding	1-3 times daily manually experimental diet - A	1-3 times daily manually commercial diet - B
Replicates	3	3
Duration	42 days	36 days
Avg. water temp.	23-24°C ambient	23-24°C ambient
Tanks	200L outdoors	200L outdoors
Weighing	every 14 days	every 14 days

In each of the two trials, fish were fed increasing amounts of feed from zero to close to maximum feed intake, referred to as zero, low, medium and high. Feed was given three times a day at the high feeding level decreasing to once daily at the low feeding level to ensure equal distribution of the food pellets among the fish.

DE and DCP content of Diet A was calculated from the DE and DCP content of the two ingredients fish meal and fish oil, as previously determined. The commercial diet, which was only available pelleted, was ground up in a hammer mill, and re-pelleted with the laboratory pellet mill after adding chromic oxide. This re-pelleted diet as a whole was used to test digestibility.

***Table 13**: Formulation and proximate analysis of two diets in Trials A and B.*

	Trial A Diet A	Trial B Diet B
Components (g kg^{-1})		
Fish meal (A)	895	
Fish meal[1]		165
Meat meal[1]		100
Soybean meal extracted[1]		420
Wheat meal[1]		222
DL-Methionine		3
Fish oil	95	50
Vitamin mix	10	10
Mineral mix		10
Di-calcium phosphate		20
Analysis (kg^{-1} DM)		
Gross energy, MJ	23.4	19.9
Crude protein, g	676	394
Crude lipid, g	199	93
Ash, g	142	108
Digestible crude protein, g	594	335
Digestible energy, MJ	21.0	15.1

[1] source Matmor Inc.

6.2.2 Results

The feed intake as well as weight gain in g $fish^{-1}$ day^{-1} of seabream fed two diets at increasing feeding levels is shown in the following Tables 14 and 15. The relationship between DE fed and energy gain (kJ $fish^{-1}$ day^{-1}) is depicted in Figure 5 and similarly the relationship between DCP fed and protein gain (g $fish^{-1}$ day^{-1}) can be seen in Figure 6.

***Table 14**: Growth performance of gilthead seabream fed increasing levels of an experimental diet in trial A (mean ± SD of three replicate tanks).*

Feeding level	Zero	Low	Medium	High
Initial BW g fish^{-1}	30.6 ± 0.2	30.0 ± 0.6	30.1 ± 0.7	29.7 ± 0.3
Final BW g fish^{-1}	25.1 ± 0.4	39.7 ± 1.7	54.8 ± 1.1	65.1 ± 1.6
Food intake g fish^{-1} day^{-1}	0.00	0.28 ± 0.01	0.65 ± 0.01	1.01 ± 0.01
Weight gain g fish^{-1} day^{-1}	-0.13 ± 0.01	0.23 ± 0.03	0.59 ± 0.03	0.84 ± 0.03
FCE		0.82 ± 0.08	0.91 ± 0.04	0.83 ± 0.03
DE intake kJ fish^{-1} day^{-1}	0.00	5.92 ± 0.17	13.57 ± 0.25	21.27 ± 0.24
Energy retention kJ fish^{-1} day^{-1}	-2.12 ± 0.28	1.51 ± 0.40	5.00 ± 0.49	8.51 ± 0.79
$k_{DE\ tot}$		0.25 ± 0.06	0.37 ±0.04	0.40 ±0.03
DCP intake g fish^{-1} day^{-1}	0.00	0.17 ± 0.00	0.39 ± 0.01	0.60 ± 0.01
Protein retention g fish^{-1} day^{-1}	-0.032 ± 0.002	0.045 ±0.007	0.109 ± 0.006	0.154 ± 0.018
$k_{DCP\ tot}$		0.27 ± 0.04	0.28 ± 0.02	0.26 ± 0.03
Lipid retention g fish^{-1} day^{-1}	-0.032 ± 0.006	0.020 ± 0.006	0.079 ± 0.007	0.143 ± 0.011

Table 15: *Growth performance of gilthead seabream fed increasing levels of a commercial diet in trial B (mean ± SD of three replicate tanks).*

Feeding level	Zero	Low	Medium*	High
Initial BW $g\ fish^{-1}$	92.1 ± 0.5	92.6 ± 1.4	92.5 91.8-93.2	93.4 ± 0.5
Final BW $g\ fish^{-1}$	81.5 ± 1.4	103.5 ± 2.8	118.9 118.9-118.9	131.1 ± 1.0
Food intake $g\ fish^{-1}\ day^{-1}$	0.00	0.74 ± 0.01	1.52 1.63-1.66	2.17 ± 0.02
Weight gain $g\ fish^{-1}\ day^{-1}$	-0.30 ± 0.03	0.30 ± 0.05	0.73 0.71-0.75	1.05 ± 0.03
FCE		0.41 ± 0.06	0.48 0.46-0.50	0.48 ± 0.01
DE intake $kJ\ fish^{-1}\ day^{-1}$	0.00	11.15 ± 0.17	23.03 22.8-23.2	32.71 ± 0.35
Energy retention $kJ\ fish^{-1}\ day^{-1}$	-5.24 ± 0.76	3.12 ± 0.65	7.45 7.33-7.53	13.08 ± 0.40
$k_{DE\ tot}$		0.28 ±0.06	0.32 0.32-0.33	0.40 ± 0.01
DCP intake $g\ fish^{-1}\ day^{-1}$	0.00	0.25 ± 0.00	0.51 0.51-0.51	0.73 ± 0.01
Protein retention $g\ fish^{-1}\ day^{-1}$	-0.087 ± 0.010	0.038 ±0.003	0.107 0.104-0.111	0.171 ± 0.014
$k_{DCP\ tot}$		0.16 ±0.01	0.21 0.20-0.22	0.24 ± 0.02
Lipid retention $g\ fish^{-1}\ day^{-1}$	-0.070 ± 0.014	0.085 ± 0.021	0.161 0.155-0.167	0.243 ± 0.005

* Fish of one tank were lost during the experiment due to technical failure.

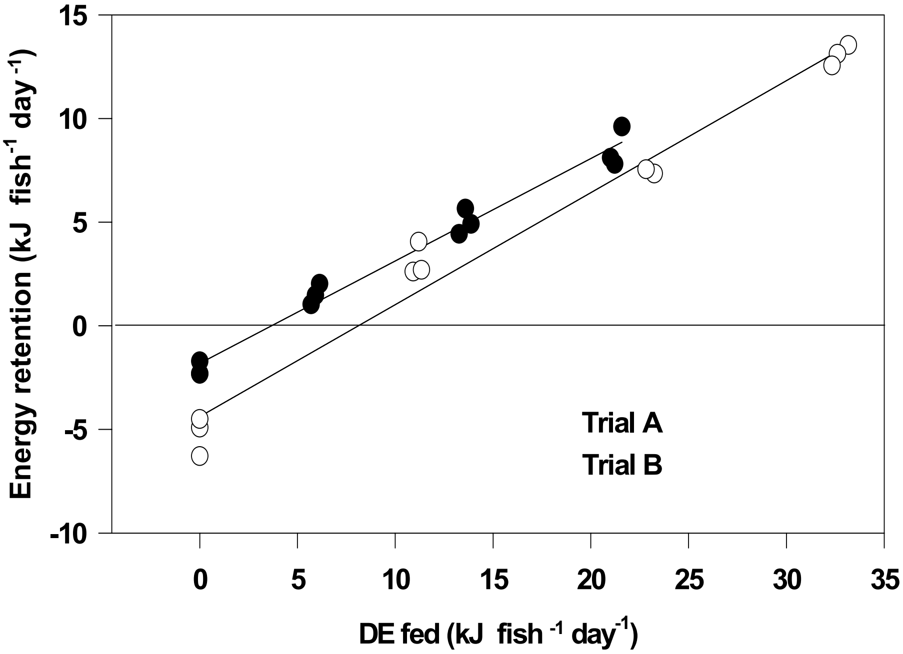

Figure 5*: Daily energy (kJ) retention per fish fed increasing levels of two different diets in Trials A and B. Each point represents 18 - 20 fish.*

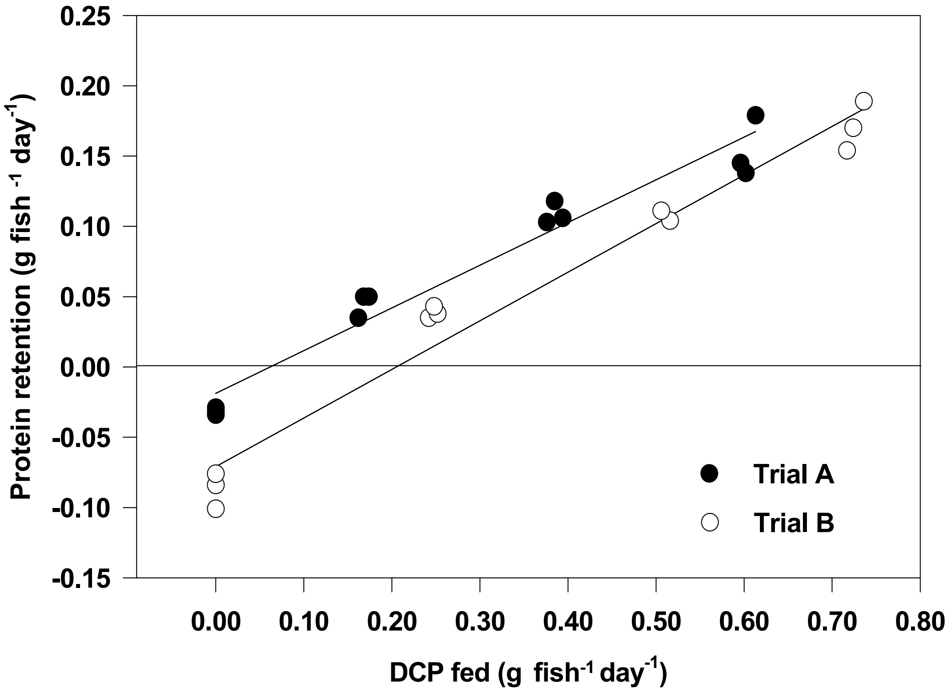

Figure 6*: Daily protein (g) retention per fish fed increasing levels of two different diets in Trials A and B. Each point represents 18 - 20 fish.*

The relationship between x = digestible energy fed (kJ) and y = energy retained (kJ) per day per fish (Figure 5) can be described by linear equations for the two diets A and B as follows:

Trial A: $y = -1.81 (\pm 0.28) + 0.49 (\pm 0.02) x$ $r^2 = 0.98$ (14)

Trial B: $y = -4.38 (\pm 0.59) + 0.54 (\pm 0.03) x$ $r^2 = 0.97$ (15)

Similar equations can be formulated for x = DCP fed (g) and y = CP retained (g) during the two respective growth trials (Figure 6):

Trial A: $y = -0.02 (\pm 0.01) + 0.30 (\pm 0.02) x$ $r^2 = 0.96$ (16)

Trial B: $y = -0.07 (\pm 0.01) + 0.35 (\pm 0.02) x$ $r^2 = 0.97$ (17)

In Table 16 the daily energy (kJ) and protein (g) retention is presented calculated per metabolic weights of $kg^{0.83}$ and $kg^{0.70}$ respectively as derived from equations (10) and **(**11).

Table 16*: Performance of gilthead seabream fed increasing levels of two different diets in trials A and B calculated per MBW of $kg^{0.83}$ and $kg^{0.70}$ for energy and protein respectively.*

	Trial A				Trial B			
Feeding level	Zero	Low	Medium	High	Zero	Low	Medium	High
Mean BW* g fish^{-1}	27.7 ±0.24	34.5 ±1.05	40.6 ±0.76	43.9 ±0.72	86.6 ±0.99	97.9 ±2.01	104.9 104.5-105.3	110.6 ±0.52
Metabolic BW $kg^{0.83}$	0.051	0.061	0.070	0.075	0.131	0.145	0.154	0.161
DE intake kJ $kg^{-0.83}$ day^{-1}		96.8 ±1.14	193.9 ±1.03	284.6 ±2.95		76.7 ±0.16	149.7 148.8-150.6	203.4 ±1.59
Energy retention kJ $kg^{-0.83}$ day^{-1}	-41.5 ±5.54	24.56 ±5.99	71.42 ±7.17	113.79 ±9.01	-39.9 ±6.15	21.44 ±4.40	48.39 47.6-49.2	81.3 ±2.33
Metabolic BW $kg^{0.70}$	0.081	0.095	0.106	0.112	0.180	0.197	0.206	0.214
DCP intake g $kg^{-0.70}$ day^{-1}		1.77 ±0.02	3.63 ±0.02	5.38 ±0.05		1.26 ±0.00	2.48 2.46-2.49	3.39 ±0.03
Protein retention g $kg^{-0.70}$ day^{-1}	-0.39 ±0.03	0.48 ±0.07	1.03 ±0.06	1.37 ±0.14	-0.48 ±0.06	0.20 ±0.02	0.52 0.50-0.54	0.80 ±0.07

*geometric mean weight (BW initial • BW final) $^{0.5}$

By expressing DE intake and the subsequent retention of energy per unit of metabolic weight ($kg^{0.83}$) the data set of the two trials A and B can be combined (Figure 7). Likewise, Figure 8 shows DCP intake and resulting protein retention per unit of metabolic weight of ($kg^{0.70}$).

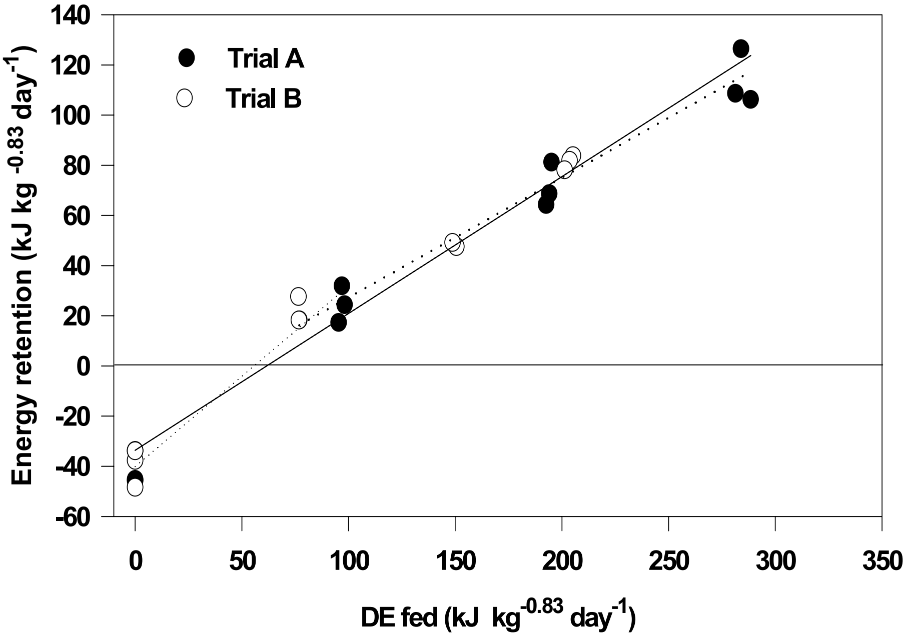

***Figure 7**: Daily energy (kJ) retention per unit metabolic weight of kg$^{0.83}$ in gilthead seabream fed increasing levels of two different diets. Each point represents 18 - 20 fish.*

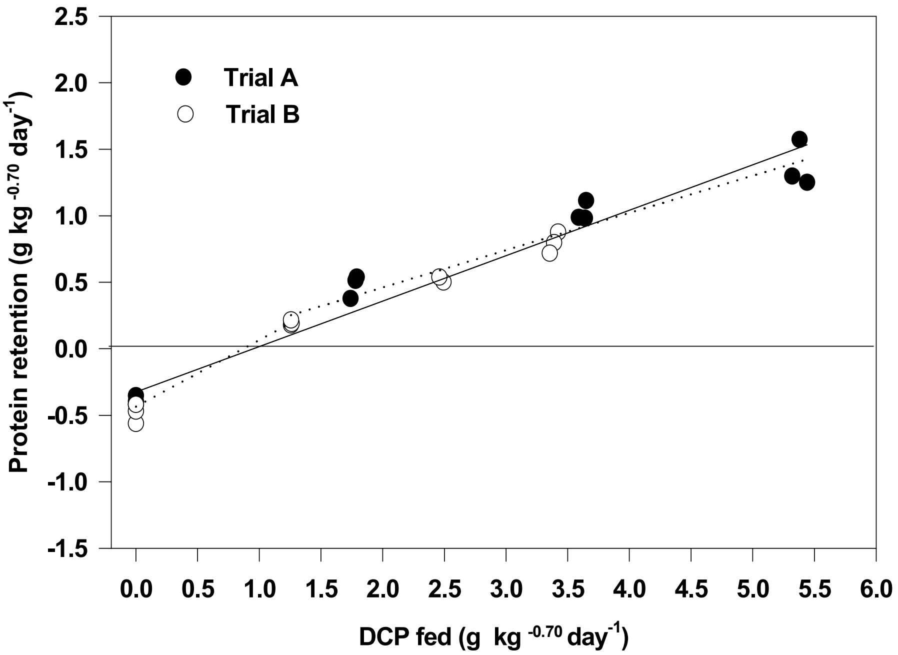

***Figure 8**: Daily protein (g) retention per unit metabolic weight of kg$^{0.70}$ in gilthead seabream fed increasing levels of two different diets. Each point represents 18 - 20 fish.*

Computing the relationship between DE fed (x) and energy retained (y) was based alternatively on one of two assumptions, namely:

i. linearity over the whole range studied, i.e. identical efficiency of utilisation of DE below and above maintenance

ii. separate calculation for levels 0 and 1 as compared to levels 1, 2 and 3, i.e. possibility of different efficiencies of utilisation of DE below and above maintenance respectively.

In parallel, the relationship between DCP fed (x) and protein retained (y) both expressed per unit of metabolic weight ($kg^{0.70}$) was based alternatively on each of the two above assumptions.

Based on assumption i the following equations were calculated:

Energy: $y = -33.65\ (\pm 3.3) + 0.55\ (\pm 0.02)\ x$ $r^2 = 0.97$ (18)

Protein: $y = -0.33\ (\pm 0.05) + 0.34\ (\pm 0.02)\ x$ $r^2 = 0.95$ (19)

For zero energy retention ($y = 0$) the required intake of DE is calculated from equation (18) as 33.65/0.55 = 61.2 kJ $BW(kg)^{-0.83}$ day^{-1}. Correspondingly, the intake of DCP attributable to maintenance is calculated from equation (19) as 0.33/0.34 = 0.97g BW $(kg)^{-0.70}$ day^{-1}. The efficiency of utilisation of DE and DCP for growth were determined as 0.55 and 0.34 respectively. Alternatively, using assumption ii the following equations were obtained:

Energy - below and at maintenance: $y = -40.2(\pm 3.1) + 0.72\ (\pm 0.05)\ x$ $r^2 = 0.95$ (20)

- above maintenance: $y = -20.5\ (\pm 5.1) + 0.48\ (\pm 0.03)\ x$ $r^2 = 0.95$ (21)

Protein - below and at maintenance: $y = -0.44\ (\pm 0.02) + 0.51\ (\pm 0.02)\ x$ $r^2 = 0.97$ (22)

- above maintenance $y = -0.10\ (\pm 0.06) + 0.28\ (\pm 0.02)\ x$ $r^2 = 0.93$ (23)

In the case of assumption ii the requirement of DE for maintenance (DE_m) would amount to 55.8 kJ $BW(kg)^{-0.83}$ day^{-1} (equation 20) and the requirement of DCP for maintenance (DCP_m) would be 0.86g $BW(kg)^{-0.70}$ day^{-1} (equation 22). The efficiencies of DE and DCP below and at maintenance are determined as 0.72 and 0.51 (equations 20 and 22) respectively. In parallel the efficiencies of DE and DCP above maintenance are determined as 0.48 and 0.28 (equations 21 and 23) respectively.

6.3 Efficiency of energy and protein utilisation as influenced by the DCP / DE ratio

6.3.1 Material and Methods

Three growth trials C, D and E each consisting of 4 to 6 dietary treatments were performed successively as described in Table 17. The fifteen diets C_{1-6}, D_{7-10} and E_{11-15} were formulated to provide a range of dietary digestible energy contents from 10 to 22 MJ kg^{-1} food and of DCP/DE ratios from 34 to 15 g MJ^{-1} (Tables 18, 19 and 20). The diets were based mainly on fish meal and fish oil, exchanging the oil with starch or cellulose where necessary to reach the required DCP to DE ranges. Fish meal as the only protein source was chosen, because of its balanced amino acid profile. Three different sources of fish meal A, B and C were used, in the course of the three trials.

Table 17*: Experimental set-up for growth trials C to E to evaluate differing dietary protein to energy ratios in seabream.*

	Trial C	Trial D	Trial E
Stocking	18 fish per tank	20 fish per tank	19 fish per tank
Initial weight (g $fish^{-1}$)	17.3	25.2	32.0
Treatment	6 diets (C1 - C6)	4 diets (D7 - D10)	5 diets (E11 - E15)
Feeding		twice daily to apparent satiation	
Replicates	3	3	3
Duration	140 days	94 days	92 days
Water temperature	21-23^{0}C ambient	22-24^{0}C ambient	22-24^{0}C ambient
Tanks	200 l outdoors	200 l outdoors	200 l outdoors
Weighing	~ every 14 days	~ every 14 days	~ every 14 days

Digestibility for all the diets C_{1-6}, D_{7-10} and E_{11-15} was determined as previously described by summing the DE and DCP values for the single ingredients used and as determined in the previous trials (Table A 15, attachment).

***Table 18**: Formulation and proximate analysis of six diets in Trial C with differing DCP/DE ratios.*

	C1	C2	C3	C4	C5	C6
Components (g kg^{-1})						
Fish meal C	620	620	620	780	780	780
Fish oil		40	85		25	65
Cellulose	360	320	275	200	175	135
Vitamins	10	10	10	10	10	10
Sipernat*	10	10	10	10	10	10
Analysis (kg^{-1} DM)						
Gross energy, MJ	19.5	20.6	21.4	20.6	20.7	22
Crude protein, g	449	447	439	547	545	539
Crude lipid, g	71	109	140	91	122	150
Ash, g	120	116	111	122	122	124
Digestible crude protein, g	370	369	362	451	450	445
Digestible energy, MJ	10.8	12.4	13.9	13.7	14.4	16.2
DCP/DE (g MJ^{-1})	34.3	29.7	26.0	32.9	31.2	27.5

*pelleting aid, DEGUSSA AG, Hanau, Germany

***Table 19**: Formulation and proximate analysis of four diets in Trial D with differing DCP/DE ratios.*

	D7	D8	D9	D10
Components (g kg^{-1})				
Fish meal A	600	735	885	600
Fish oil	95	95	95	163
Cornstarch	285	150		150
Cellulose				67
Vitamins	10	10	10	10
Sipernat	10	10	10	10
Analysis (kg^{-1} DM)				
Gross energy, MJ	21.8	22.3	23.4	23.4
Crude protein, g	460	559	676	463
Crude lipid, g	173	191	199	238
Ash, g	107	132	142	105
Digestible crude protein, g	405	492	595	407
Digestible energy, MJ	18.5	19.5	21.1	19.6
DCP/DE (g MJ^{-1})	21.9	25.2	28.2	20.8

***Table 20**: Formulation and proximate analysis of five diets in Trial E with differing DCP/DE ratios.*

	E11	E12	E13	E14	E15
Components (g kg^{-1})					
Fish meal B	870	805	750	700	650
Fish oil	100	150	200	250	300
Vitamins	10	10	10	10	10
Sipernat	20	35	40	40	40
Analysis (kg^{-1} DM)					
Gross energy, MJ	22.6	23.3	24	24	24.9
Crude protein, g	560	512	470	444	406
Crude lipid, g	189	240	272	332	371
Ash, g	138	143	148	158	157
Digestible crude protein, g	493	451	414	391	357
Digestible energy, MJ	20.2	20.9	21.7	21.8	22.8
DCP/DE (g MJ^{-1})	24.4	21.5	19	17.9	15.7

To test the influence of energy level on feed intake fish were fed to apparent satiation twice a day by the same person throughout the experiments. The point of satiation was determined when the feed pellets started to reach the bottom of the tanks, and feeding activity declined. Using this technique feed waste could be prevented as the tanks were visible till the bottom. Voluntary feed intake was recorded daily.
Ten fish from each Trial C to E were sampled for analysis at the beginning, and at the end of the experiments fish were collected from each tank and immediately frozen.

6.3.2 Results

The feed intake as well as weight gain in g $fish^{-1}$ day^{-1} of seabream fed 15 diets varying in protein to energy ratios is shown in Tables 21, 22 and 23.

Table 21: *Growth performance of gilthead seabream fed six diets with differing protein to energy ratios in Trial C (mean ± SD).*

	C1	C2	C3	C4	C5	C6
Initial BW (g fish^{-1} day^{-1})	17.04 ± 0.07	17.45 ± 0.30	17.65 ± 0.78	17.96 ± 0.25	17.39 ± 0.16	17.24 ± 0.58
Final BW (g fish^{-1} day^{-1})	41.92 ± 0.53	49.94 ± 3.56	58.54 ± 4.27	70.56 ± 4.04	71.28 ± 2.83	70.65 ± 1.85
Food intake(g fish^{-1} day^{-1})	0.38 ± 0.01	0.46 ± 0.04	0.57 ± 0.03	0.55 ± 0.02	0.58 ± 0.02	0.58 ± 0.01
Weight gain (g fish^{-1} day^{-1})	0.18 ± 0.00	0.23 ± 0.02	0.29 ± 0.03	0.37 ± 0.03	0.38 ± 0.020	0.38 ± 0.02
FCE	0.47 ±0.00	0.50 ±0.04	0.52 ±0.03	0.68 ±0.03	0.67 ±0.02	0.66 ±0.04
DE intake (kJ fish^{-1} day^{-1})	4.11 ± 0.05	5.72 ± 0.50	7.88 ± 0.44	7.55 ± 0.31	8.33 ± 0.22	9.41 ± 0.17
Energy retention (kJ fish^{-1} day^{-1})	0.99 ± 0.05	1.58 ± 0.22	2.27 ± 0.21	2.65 ± 0.31	3.09 ± 0.30	3.21 ± 0.25
k_{DEtot}	0.24 ±0.01	0.28 ±0.02	0.029 ±0.01	0.35 ±0.05	0.37 ±0.03	0.34 ±0.03
DCP intake (g fish^{-1} day^{-1})	0.141 ± 0.002	0.170 ± 0.015	0.205 ± 0.011	0.249 ± 0.010	0.260 ± 0.007	0.258 ± 0.005
Protein retention (g fish^{-1} day^{-1})	0.029 ± 0.000	0.042 ± 0.003	0.052 ±0.001	0.070 ±0.004	0.070 ±0.006	0.071 ± 0.004
k_{DCPtot}	0.21 ±0.00	0.25 ±0.02	0.25 ±0.01	0.28 ±0.01	0.27 ±0.02	0.28 ±0.02
Lipid retention (g fish^{-1} day^{-1})	0.010 ±0.001	0.018 ±0.004	0.026 ±0.004	0.028 ±0.004	0.037 ±0.004	0.041 ±0.002

Table 22: *Growth performance of gilthead seabream fed four diets with differing protein to energy ratios in Trial D (mean ± SD).*

	D7	D8	D9	D10
Initial BW (g $fish^{-1}$ day^{-1})	25.14 ± 0.19	25.47 ± 0.09	25.33 ± 0.47	25.40 ± 0.26
Final BW (g $fish^{-1}$ day^{-1})	77.19 ± 0.41	89.17 ±1.2	95.25 ± 1.31	93.50 ± 0.58
Food intake(g $fish^{-1}$ day^{-1})	0.82 ± 0.01	0.85 ± 0.01	0.85 ± 0.02	0.85 ± 0.01
Weight gain(g $fish^{-1}$ day^{-1})	0.55 ± 0.01	0.68 ± 0.01	0.74 ± 0.01	0.72 ± 0.00
FCE	0.67 ±0.02	0.79 ±0.01	0.88 ±0.02	0.85 ±0.01
DE intake(kJ $fish^{-1}$ day^{-1})	15.66 ± 0.21	16.81 ± 0.12	17.84 ± 0.50	16.74 ± 0.16
Energy retention (kJ $fish^{-1}$ day^{-1})	5.52 ± 0.22	6.73 ± 0.33	7.19 ± 0.14	7.35 ± 0.00
k_{DEtot}	0.35 ±0.02	0.40 ±0.02	0.40 ±0.02	0.44 ±0.00
DCP intake(g $fish^{-1}$ day^{-1})	0.334 ± 0.005	0.420 ± 0.003	0.503 ± 0.014	0.348 ± 0.003
Protein retention(g $fish^{-1}$ day^{-1})	0.094 ± 0.001	0.120 ± 0.003	0.134 ± 0.001	0.125 ± 0.004
k_{DCPtot}	0.28 ±0.00	0.28 ±0.00	0.27 ±0.00	0.36 ±0.01
Lipid retention(g $fish^{-1}$ day^{-1})	0.090 ±0.005	0.111 ±0.008	0.120 ±0.007	0.131 ±0.005

__Table 23:__ Growth performance of gilthead seabream fed five diets with differing protein to energy ratios in Trial E (mean ± SD).

	E11	E12	E13	E14	E15
Initial BW (g $fish^{-1}$ day^{-1})	31.61	32.14	32.66	31.58	31.71
	±0.17	± 0.36	± 0.48	± 0.21	± 0.24
Final BW (g $fish^{-1}$ day^{-1})	104.28	109.48	102.41	99.48	94.59
	± 3.95	± 4.54	± 5.90	± 5.16	± 4.97
Food intake (g $fish^{-1}$ day^{-1})	0.93	0.96	0.94	0.86	0.82
	± 0.07	± 0.09	± 0.07	± 0.06	± 0.05
Weight gain (g $fish^{-1}$ day^{-1})	0.79	0.84	0.76	0.74	0.68
	± 0.04	± 0.05	± 0.07	± 0.06	± 0.06
FCE	0.85	0.87	0.80	0.86	0.83
	±0.02	±0.03	±0.03	±0.03	±0.02
DE intake (kJ $fish^{-1}$ day^{-1})	18.78	20.17	20.48	18.64	18.71
	± 1.51	± 1.88	± 1.07	± 1.25	± 1.22
Energy retention (kJ $fish^{-1}$ day^{-1})	7.67	8.05	7.61	7.66	7.40
	± 0.55	± 0.66	± 0.60	± 0.84	± 0.52
k_{DEtot}	0.41	0.40	0.37	0.41	0.40
	±0.00	±0.01	±0.01	±0.02	±0.01
DCP intake (g $fish^{-1}$ day^{-1})	0.458	0.435	0.391	0.334	0.293
	± 0.037	± 0.041	± 0.044	± 0.022	± 0.019
Protein retention (g $fish^{-1}$ day^{-1})	0.145	0.148	0.127	0.118	0.108
	± 0.012	± 0.009	± 0.015	± 0.010	± 0.011
k_{DCPtot}	0.32	0.34	0.33	0.35	0.37
	±0.01	±0.01	±0.01	±0.01	±0.01
Lipid retention (g $fish^{-1}$ day^{-1})	0.121	0.133	0.131	0.135	0.144
	±0.013	±0.020	±0.017	±0.013	±0.010

As fish in the three trials C, D and E had different weights initially, feed intake and weight gain were expressed in g $BW(kg)^{-0.70}$ day^{-1} to correct for the influence of fish size on feed consumption. Feed intake of seabream along the growth period has been shown to relate to a body weight with an exponent of $kg^{0.70}$ when feeding on the commercial diet (chapter 3.3.1, equation 4). Intake and retention of energy and protein were calculated per MBW of $kg^{0.83}$ and $kg^{0.70}$ respectively, as has been done in trials A and B. The data are shown for trials C, D and E in Tables 24, 25 and 26.

Table 24: *Growth performance of gilthead seabream fed six diets with differing protein to energy ratios in Trial C calculated per MBW of* $kg^{0.83}$ *and* $kg^{0.70}$ *for energy and protein respectively (mean ± SD).*

	C1	C2	C3	C4	C5	C6
Mean BW (g $fish^{-1}$)	26.72 ±0.11	29.5 ±1.30	32.14 ±1.84	35.59 ±1.19	35.20 ±0.81	34.89 ±0.26
Metabolic BW $(kg)^{0.70}$	0.079	0.085	0.090	0.097	0.096	0.095
Metabolic BW $(kg)^{0.83}$	0.049	0.054	0.058	0.063	0.062	0.062
Food intake (g $kg^{-0.70}$ day^{-1})	4.81 ±0.05	5.43 ±0.35	6.29 ±0.22	5.69 ±0.12	6.02 ±0.09	6.08 ±0.09
Weight gain (g $kg^{-0.70}$ day^{-1})	2.24 ±0.05	2.73 ±0.19	3.24 ±0.15	3.88 ±0.19	4.01 ±0.14	3.99 ±0.19
DE intake (kJ $kg^{-0.83}$ day^{-1})	83.15 ±0.85	106.41 ±6.48	136.82 ±4.80	120.35 ±2.14	133.94 ±1.85	152.39 ±2.12
Energy retention (kJ $kg^{-0.83}$ day^{-1})	20.06 ±1.00	29.43 ±3.17	39.27 ±2.73	42.31 ±5.47	49.71 ±3.89	52.07 ±4.17
DCP intake (g $kg^{-0.70}$ day^{-1})	1.78 ±0.02	2.00 ±0.13	2.28 ±0.08	2.57 ±0.05	2.71 ±0.04	2.71 ±0.04
Protein retention (g $kg^{-0.70}$ day^{-1})	0.37 ±0.00	0.49 ±0.02	0.57 ±0.01	0.72 ±0.03	0.73 ±0.05	0.75 ±0.05

Table 25*: Growth performance of gilthead seabream fed four diets with differing protein to energy ratios in Trial D calculated per MBW of $kg^{0.83}$ and $kg^{0.70}$ for energy and protein respectively (mean ± SD).*

	D7	D8	D9	D10
Mean BW (g $fish^{-1}$)	44.05 ±0.18	47.66 ±0.39	49.11 ±0.78	48.74 ±0.41
Metabolic $BWkg^{0.70}$	0.112	0.119	0.121	0.121
Metabolic $BWkg^{0.83}$	0.075	0.080	0.082	0.081
Food intake (g $kg^{-0.70}$ day^{-1})	7.33 ±0.10	7.19 ±0.05	6.97 ±0.13	7.08 ±0.04
Weight gain (g $kg^{-0.70}$ day^{-1})	4.93 ±0.05	5.71 ±0.07	6.13 ±0.03	6.01 ±0.03
DE intake (kJ $kg^{-0.83}$ day^{-1})	209.03 ±2.80	210.30 ±1.65	217.61 ±3.88	205.56 ±1.09
Energy retention (kJ $kg^{-0.83}$ day^{-1})	73.67 ±2.82	84.13 ±4.49	87.68 ±2.72	90.23 ±0.64
DCP intake (g $kg^{-0.70}$ day^{-1})	2.97 ±0.04	3.54 ±0.03	4.15 ±0.08	2.88 ±0.02
Protein retention (g $kg^{-0.70}$ day^{-1})	0.83 ±0.01	1.01 ±0.02	1.11 ±0.00	1.04 ±0.03

Table 26: Growth performance of gilthead seabream fed five diets with differing protein to energy ratios in Trial E calculated per MBW of $kg^{0.83}$ and $kg^{0.70}$ for energy and protein respectively (mean ± SD).

	E11	E12	E13	E14	E15
Mean BW (g $fish^{-1}$)	57.41 ±1.18	59.30 ±0.93	57.84 ±1.20	56.02 ±1.44	54.75 ±1.23
Metabolic BW $kg^{0.70}$	0.135	0.138	0.136	0.133	0.131
Metabolic BW $kg^{0.83}$	0.093	0.096	0.094	0.091	0.090
Food intake (g $kg^{-0.70}$ day^{-1})	6.87 ±0.46	6.97 ±0.57	6.94 ±0.38	6.43 ±0.34	6.27 ±0.31
Weight gain (g $kg^{-0.70}$ day^{-1})	5.84 ±0.23	6.07 ±0.31	5.57 ±0.42	5.54 ±0.32	5.22 ±0.35
DE intake (kJ $kg^{-0.83}$ day^{-1})	201.11 ±12.96	210.20 ±16.84	218.12 ±7.93	203.73 ±10.48	208.40 ±9.68
Energy retention (kJ $kg^{-0.83}$ day^{-1})	82.12 ±4.63	83.93 ±5.84	81.06 ±4.88	83.68 ±7.40	82.44 ±4.36
DCP intake (g $kg^{-0.70}$ day^{-1})	3.39 ±0.23	3.14 ±0.26	2.87 ±0.28	2.51 ±0.14	2.24 ±0.11
Protein retention (g $kg^{-0.70}$ day^{-1})	1.07 ±0.08	1.07 ±0.05	0.93 ±0.09	0.89 ±0.06	0.82 ±0.07

As the amount of feed consumed is one of the most important factors for high weight gains, one of the first objectives was to test whether voluntary feed intake was controlled by energy or protein levels in the diet. Figure 9 describes the relationships between digestible energy content of the food and the daily voluntary feed intake of fish per $kg^{0.70}$ of body weight. The resulting curve shows low feed intake (y, g $kg^{-0.70}day^{-1}$) at low dietary DE density, which increased with increasing DE and decreased after reaching a maximum (Figure 9, equation 24).

$$y = - 5.07 (\pm 1.49) + 1.25 (\pm 0.18)\, x - 0.032 (\pm 0.005)\, x^2 \qquad r^2 = 0.76 \qquad (24)$$

No direct relationship could be established between protein content of the feed and appetite of fish (Figure 10). Consequently the absolute amount of DE and DCP the fish is consuming per $kg^{0.83}$ and $kg^{0.70}$ body weight respectively is shown in Figure 11a.

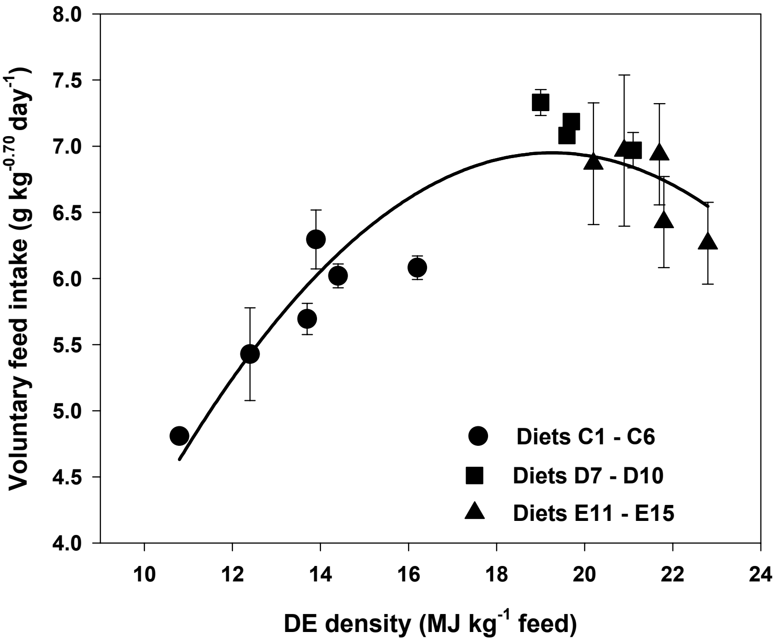

***Figure 9**: Voluntary daily feed intake (g kg$^{-0.70}$ day^{-1}) of gilthead seabream relative to the digestible energy content of 15 feeds differing in DE and DCP contents. Each point represents mean ± SD of triplicate treatments.*

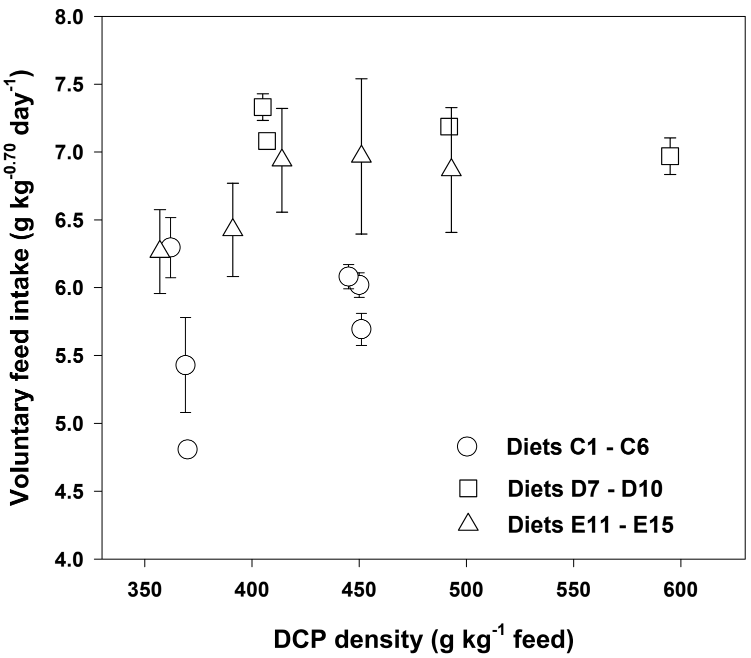

Figure 10: Voluntary daily feed intake (g kg$^{-0.70}$ day^{-1}) of gilthead seabream relative to the digestible crude protein content of 15 feeds differing in DE and DCP contents. Each point represents mean ± SD of triplicate treatments.

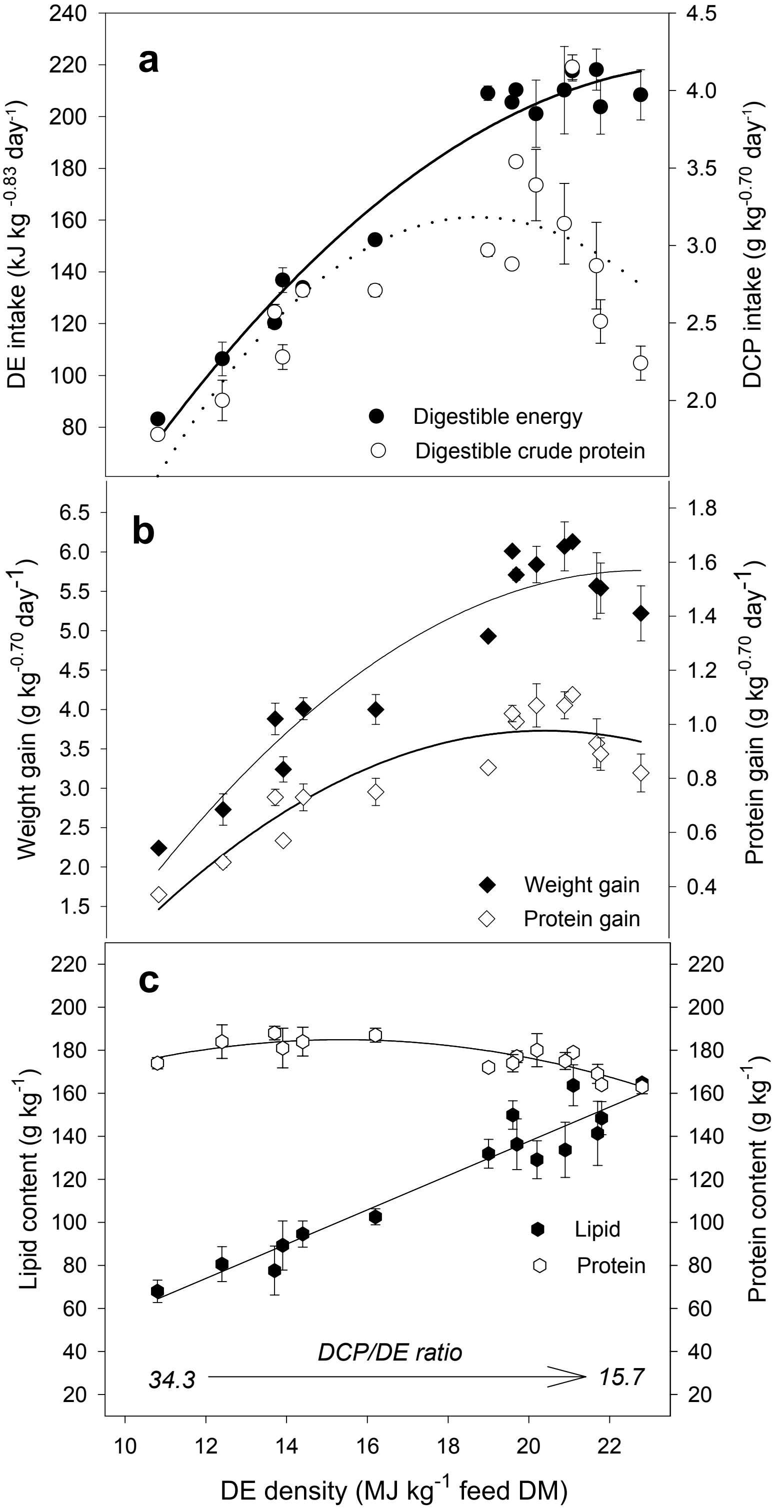

Figure 11a, b, c: *Daily intake of DE and DCP (a) per metabolic weights of $kg^{0.83}$ and $kg^{0.70}$, (b) weight and protein gain (g $kg^{-0.70}$ day^{-1}) and protein and lipid retention (c) per unit weight gain (g kg^{-1}) of gilthead seabream fed fifteen diets varying in DE content (MJ kg^{-1}). Each point represents mean ± SD of triplicate treatments.*

The amount of DE consumed increased to approach a maximum, however, the DCP intake decreased at high DE density (Figure 11a). The pattern of voluntary DE intake (y, kJ day^{-1} kg$^{-0.83}$) can be expressed by an exponential curve with the following general equation:

$y =$ a • [1 - e $^{(-b (x - c))}$]

where

a = 282 ± 29.9 (25)

b = 0.102 ± 0.027

c = 7.69 ± 0.68

$r^2 = 0.94$

The resulting DCP intake (y, g kg$^{-0.70}$ day^{-1}) follows a quadratic curve (Figure 11a) with parameters as shown below:

$y = - 6.10 (\pm1.66) + 0.99 (\pm0.20) x - 0.026 (\pm0.006) x^2$ $r^2 = 0.55$ (26)

The course of actual weight and protein gain (g kg$^{-0.70}$ day^{-1}) as a result of the dietary DE and DCP intake are presented in Figure 11b. It is obvious, that at low energy and protein intake, growth as well as feed conversion efficiency were low (see also Table 17), but both increased gradually with increasing feed intake. At high DE weight and protein gain started to decline again in direct response to the lower DCP intake (Figure 11b).

Weight and protein gain in relation to dietary DE density can be best described by second order polynomial equations as shown below:

Weight gain (g kg$^{-0.70}$ day^{-1}) =

$- 7.84 (\pm1.72) + 1.19 (\pm0.21) x - 0.026 (\pm0.006) x^2$ $r^2 = 0.89$ (27)

Protein gain (g kg$^{-0.70}$ day^{-1}) =

$- 1.96 (\pm0.38) + 0.287 (\pm0.047) x - 0.007 (\pm0.001) x^2$ $r^2 = 0.82$ (28)

As a result of feeding diets with varying DE to DCP contents the composition of the weight gain of the fish fed the 15 experimental diets changed (Figure 11c). Protein gain per kg weight gain showed narrow limits between 158 and 187g, with an indication of an decline at high DE density, whereas lipid gain showed wide ranges from 55 up to 210g kg^{-1} with increasing DE intake, without reaching a maximum.

Figure 12 shows the relationship between digestible energy intake and energy gain referring to the metabolic weight of kg$^{0.83}$. The relationship between dietary DE intake (x, kJ kg$^{-0.83}$) and energy retained (y, kJ $kg^{-0.83}$) per day was found to be linear as well as seen in Figure 7 and can be described by the following formula:

$$y = - 21.2\ (\pm 3.51) + 0.50\ (\pm 0.019)\ x \qquad r^2 = 0.94 \qquad (29)$$

Thus the partial efficiency of DE for growth above maintenance as defined by the slope of the curve is 0.50.

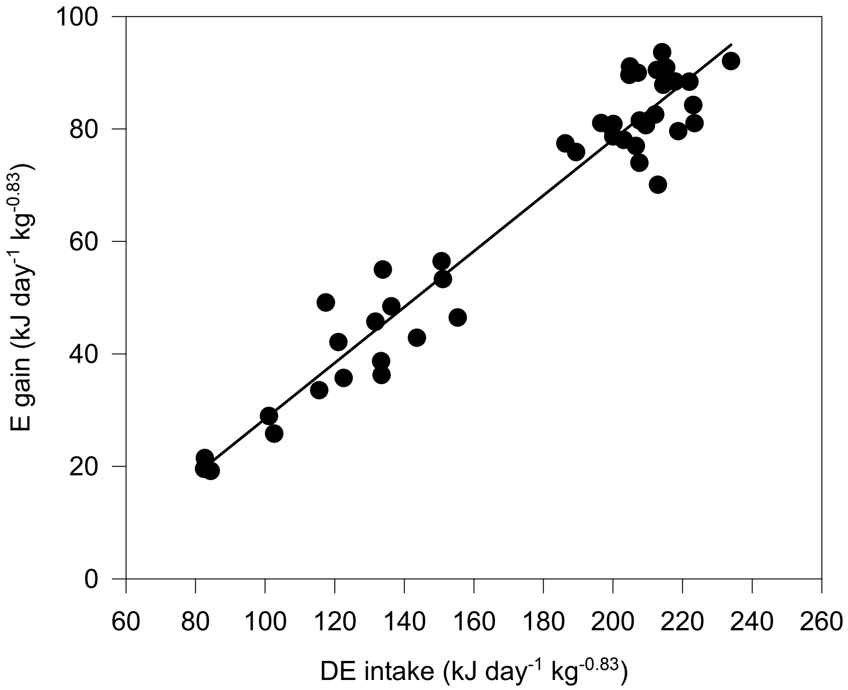

Figure 12: *Daily energy retention per unit metabolic body weight of kg$^{0.83}$ in gilthead seabream for trials C, D and E fed diets differing in DE and DCP content. Each point represents 18 - 20 fish.*

Figure 13 depicts the relationship between DCP intake and protein gain per metabolic weight of $kg^{0.70}$. Assuming a linear relationship between dietary DCP intake (x, g $kg^{-0.70}$) and protein retained (y, g $kg^{-0.70}$) per day would result in an equation as follows:

$$y = -0.033\ (\pm 0.083) + 0.31\ (\pm 0.029)\ x \qquad r^2 = 0.72 \qquad (30)$$

Presented as an exponential curve with the following general equation

$y = a \cdot [1 - e^{(-b\,(x - c))}]$

however increased the regression coefficient R^2 from 0.72 to 0.79 as seen in equation (31) and Figure 13

where

a = 1.26 ± 0.13 (maximum protein gain in g $kg^{-0.70}$ day^{-1}) (31)

b = 0.81 ± 0.23

c = 1.32 ± 0.14

r^2 = 0.79

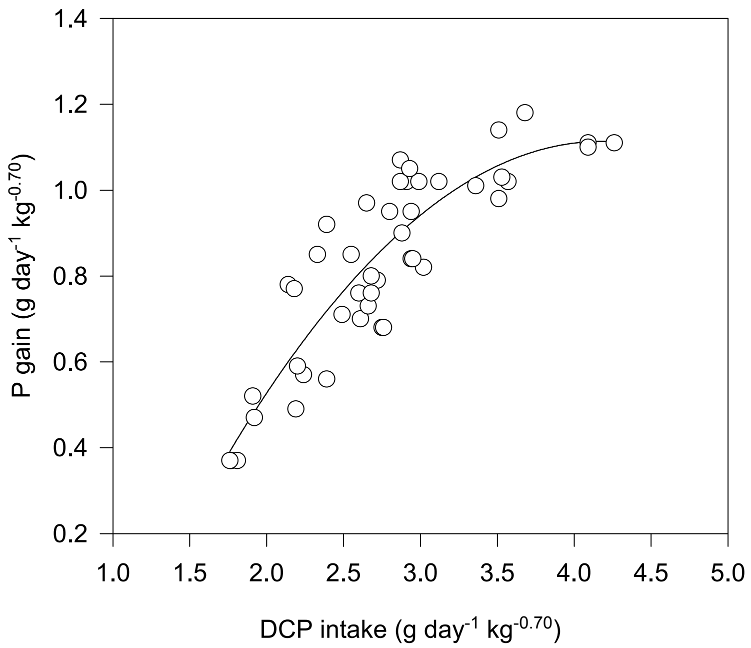

***Figure 13**: Daily protein retention per unit metabolic body weight of $kg^{0.70}$ in gilthead seabream for trials C, D and E fed diets differing in DE and DCP content. Each point represents 18 - 20 fish.*

Combining the data set from Trials A and B to increase the range of DE and DCP intake (Figures 14 and 15) confirms the same pattern: The relationship between dietary DE intake (x, kJ $kg^{-0.83}$) and energy retained (y, kJ $kg^{-0.83}$) per day for the combined data over a wide range of energy intake was linear as well as seen in Figure 14.

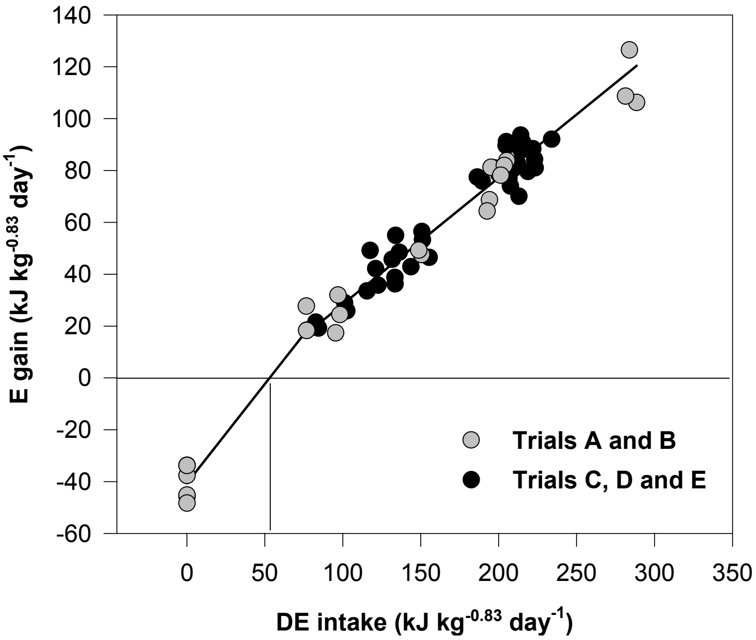

Figure 14: *Daily energy retention per unit metabolic body weight of kg$^{0.83}$ in gilthead seabream fed various diets differing in DE and DCP content. Points combined data set from Trials A, B, C, D and E . Each point represents 18 - 20 fish.*

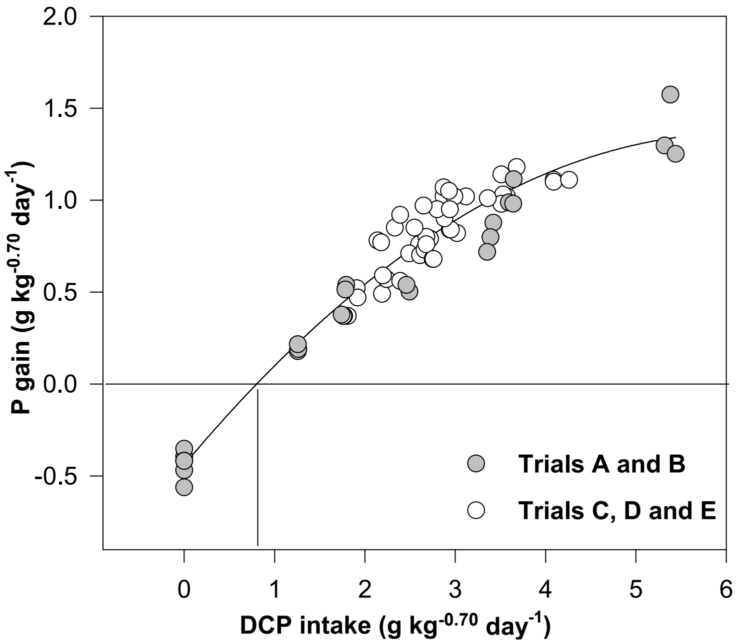

Figure 15: *Daily protein retention per unit metabolic body weight kg$^{0.70}$ in gilthead seabream fed various diets differing in DE and DCP content. Points combined data set from Trials A, B, C, D and E . Each point represents 18 - 20 fish.*

Based on assumption i linearity over the whole range as mentioned before, the following formula can be established:

Energy: $y = -30.30\ (\pm 2.27) + 0.54\ (\pm 0.01)\ x$ $r^2 = 0.96$ (32)

which results in a requirement of DE for maintenance (DE_m) of 56.1 kJ $BW(kg)^{-0.83}$ day^{-1} and an efficiency of 0.54.

Alternatively, using assumption ii the following equations were obtained:

Energy - below and at maintenance: $y = -40.61\ (\pm 2.78) + 0.76\ (\pm 0.05)\ x$ $r^2 = 0.97$ (33)

- above maintenance: $y = -20.38\ (\pm 3.01) + 0.49\ (\pm 0.02)\ x$ $r^2 = 0.94$ (34)

In the case of assumption ii the requirement of DE for maintenance (DE_m) would amount to 53.43 kJ $BW(kg)^{-0.83}$ day^{-1} (equation 33) The efficiencies of DE below and at maintenance are determined as 0.76 (equation 33) and the efficiency of DE above maintenance as 0.49 (equation 34).

The relationship between dietary DCP intake (x, g $kg^{-0.70}$) and protein retained (y, g $kg^{-0.70}$) per day for the whole data set shows even a more pronounced non-linearity (Figure 15) and the exponential curve shows a maximum protein retention of 1.89g $kg^{-0.70}$ day^{-1}.

The following exponential curve can be formulated

$y = a \cdot [1 - e^{(-b\,(x - c))}]$

where

a = 1.89 ± 0.20 (maximum protein gain in g $kg^{-0.70}$ day^{-1}) (35)

b = 0.28 ± 0.04

c = 0.77 ± 0.05

$r^2 = 0.94$

In this case DCP_m is defined by the point of zero protein gain which is found at an intake of 0.77g $kg^{-0.70}$ day^{-1}.

To summarise the latest results the following values could be determined:

DE_m = 53.4 kJ $BW(kg)^{-0.83}$ day^{-1}

the efficiency of DE for growth $k_{DEg} = 0.49$

DCP_m = 0.77 g $kg^{-0.70}$ day^{-1}.

6.4 Optimal protein utilisation

Due to the dual utilisation of protein for protein accretion as well as an energy source, it is difficult to define the efficiency of seabream for protein retention alone. In the following two approaches are suggested:

a) using interpolation to estimate the optimal value of k_{DCPg}.

b) using multiple regression analysis to estimate efficiencies for protein (k_P) and lipid (k_L) deposition simultaneously.

6.4.1 Interpolation

As the relationship between protein intake and protein retained is not linear as seen in Figure 15, one constant value for the coefficient of protein utilisation cannot be established. To estimate the various coefficients for protein utilisation for fish fed each of the 15 diets with varying DCP to DE supply the efficiency of protein retention above maintenance was calculated in the following manner:

$$k_{DCPg} = \text{Protein gain (g kg}^{-0.70}\text{ day}^{-1}) / [\text{DCP fed (g kg}^{-0.70}\text{ day}^{-1}) - DCP_m\text{ (g kg}^{-0.70}\text{ day}^{-1})] \quad (36)$$

Thus the partial efficiency of protein utilisation for growth above maintenance is shown below in Table 27 (see Table A20 attachment as well).

***Table 27**: Calculated values for partial efficiency of protein utilisation for growth - k_{DCPg} using the value DCP_m of 0.77g kg$^{-0.70}$ day^{-1} for diets from Trials C to D (mean ± SD).*

	k_{DCPg}
C1	0.37 ± 0.01
C2	0.40 ± 0.06
C3	0.38 ± 0.03
C4	0.40 ± 0.02
C5	0.38 ± 0.03
C6	0.39 ± 0.04
D7	0.38 ± 0.01
D8	0.37 ± 0.01
D9	0.33 ± 0.01
D10	0.49 ± 0.02
E11	0.41 ± 0.02
E12	0.45 ± 0.03
E13	0.44 ± 0.02
E14	0.51 ± 0.03
E15	0.56 ± 0.01

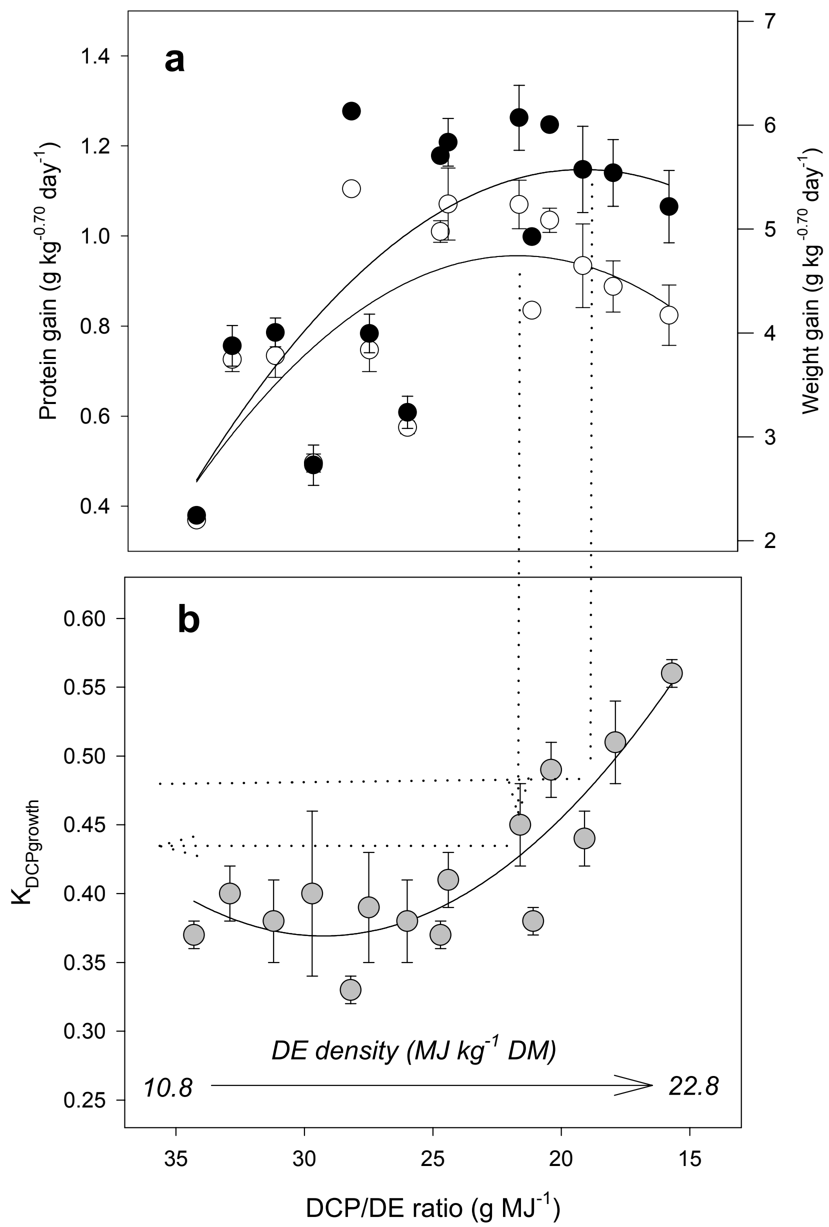

***Figure 16 a, b**: Relationship between protein efficiency values for growth (k_{DCPg}) and dietary DCP/DE ratio for gilthead seabream fed fifteen experimental diets with varying DE and DCP contents in Trials C , D and E (mean ± SD).*

The efficiency of protein utilisation calculated for the 15 diets covered a wide range between 0.33 up to 0.56 as seen in Table 27. Figure 16 a, b illustrates the relationship of the various values for k_{DCPg} (y) with changing DCP/DE ratios (x). At high DCP/DE ratios, which correspond to low dietary energy levels in the trials, efficiency was lowest only to increase with decreasing DCP/DE ratios until a maximum of 0.56. This curve could be fitted to a power function with the following equation:

$y = 1.24\ (\pm 0.12) - 0.059\ (\pm 0.009)\ x + 0.001\ (\pm 0.0002)\ x^2$ $r^2 = 0.70$ (37)

Protein gain dependent on DCP/DE ratio was
$y = -0.513\ (\pm 0.536) + 0.136\ (\pm 0.044)\ x - 0.0031\ (\pm 0.0009)\ x^2$ $r^2 = 0.46$ (38)

Weight gain dependent on DCP/DE ratio was
$y = 0.834\ (\pm 2.8) + 0.497\ (\pm 0.233)\ x - 0.013\ (\pm 0.0046)\ x^2$ $r^2 = 0.54$ (39)

According to Figure 16 a, b maximum protein gain was achieved were k_{DCPg} was around 0.43, but a higher value of protein utilisation of 0.48 could be found for maximum weight gain.

6.4.2 Multiple regression analysis

To determine the utilisation of dietary energy for protein and lipid deposition simultaneously, multiple regression analysis was employed. In the first step, by arranging the relationship according to the common equation for quantification of energy requirement in fish, DE intake is made the dependent variable (*y*) and energy gain (*x*) the independent variable:

$$\text{DE intake (kJ kg}^{-0.83}\text{ day}^{-1}) = DE_m + 1/k_{PL} \cdot \text{energy gain (kJ kg}^{-0.83}\text{ day}^{-1}) \quad (40)$$

Plotting this linear regression results in the maintenance requirement DE_m as the intercept and the slope $1/k_{PL}$ - the reciprocal of k_{PL} - describes the requirement of DE (kJ) per unit of energy deposited (kJ). The individual values obtained for DE_m and $1/k_{PL}$ for seabream are summarised in Table 28. As energy retention as a whole consists predominantly of protein and lipid, efficiency can be determined for protein and lipid simultaneously by using a multiple regression analysis, as described first by Kielanowski (1965). Based on the above equation for DE intake (*y*) and the same data base, a multiple regression can be established where both protein and lipid gain (in kJ $kg^{-0.80}$ day^{-1}) are expressed as their energy equivalents using 23.6 kJ g^{-1} protein (*x*) and 39 kJ g^{-1} lipid (*z*) retention, respectively.

$$\text{DE intake (kJ kg}^{-0.83}\text{ day}^{-1}) =$$
$$DE_m + 1/k_P \bullet \text{protein retention (kJ kg}^{-0.83}\text{ day}^{-1}) + 1/k_L \bullet \text{lipid retention (kJ kg}^{-0.83}\text{ day}^{-1}) \quad (41)$$

As described in Table 28, a number of scenarios can be exercised in establishing those values depending whether using the whole data set, or excluding the non-fed groups from Trials A and B.

Including the zero groups means linearity over the whole range according to assumption i as mentioned before, and excluding the zero groups means separate efficiency of utilisation for growth above maintenance according to assumption ii.

***Table 28**: Constants for maintenance requirement (DE_m) and energy requirement for protein ($1/k_P$) and lipid deposition ($1/k_L$) derived from linear regression analysis (values ± SE) and using two assumptions i and ii.*

Type of regression	DE_m	$1/k_{PL}$	$1/k_P$	$1/k_L$	r^2	Remarks
$y = DE_m + 1/k_{PL} \cdot x$	59.82 (±2.91)	1.78 (±0.04)	-	-	0.96	assumption i
$y = DE_m + 1/k_{PL} \cdot x$	46.52 (±4.17)	1.97 (±0.06)	-	-	0.95	assumption ii
$y = DE_m + 1/k_P \cdot x + 1/k_L \cdot z$	54.74 (±3.18)	-	1.80 (±0.23)	1.67 (±0.14)	0.96	assumption i
$y = DE_m + 1/k_P \cdot x + 1/k_L \cdot z$	41.14 (±5.14)	-	2.27 (±0.27)	1.65 (±0.13)	0.94	assumption ii

The values in Table 28 were derived by expressing DE intake and retention in units per MBW of $(kg)^{0.83}$. Another general model can be established according to

$$DE\ (kJ\ fish^{-1}\ day^{-1}) = a\ BW(kg)^{b} + 1/k_P \cdot \text{protein retention}\ (kJ\ fish^{-1}\ day^{-1}) + 1/k_L \cdot \text{lipid retention}\ (kJ\ fish^{-1}\ day^{-1}) \quad (42)$$

where all the constants a, b, c and d are estimated by least square principles and DE intake as well as retention is expressed in kJ per fish per day. Additional to the feature of including or excluding the non-fed groups, the exponent for the metabolic body weight $(kg)^b$ can be selected or set at a fixed value of $(kg)^{0.83}$ as determined in this study with seabream (Table 29).

Table 29: *Constants for maintenance requirement (DE_m) and energy requirement for protein (k_P) and lipid deposition (k_L) derived from non - linear analysis (values ± SE).*

Type of regression	a	b	$1/k_{PL}$	$1/k_P$	$1/k_L$	r^2	Remarks
$y = a \cdot (kg)^b + 1/k_{PL} \cdot x$	50.10 (±9.06)	0.77 (±0.065)	1.77 (±0.041)	-	-	0.98	assumption i
$y = a \cdot (kg)^{0.83} + 1/k_{PL} \cdot x$	59.37 (±10.80)	0.83 (±0.066)	1.78 (±0.041)	-	-	0.98	assumption i
$y = a \cdot (kg)^b + 1/k_{PL} \cdot x$	33.33 (±8.17)	0.74 (±0.080)	2.00 (±0.057)	-	-	0.98	assumption ii
$y = a \cdot (kg)^{0.83} + 1/k_{PL} \cdot x$	42.57 (±10.15)	0.83 (±0.082)	2.01 (±0.058)	-	-	0.98	assumption ii
$y = a \cdot (kg)^b + 1/k_P \cdot x + 1/k_L \cdot z$	41.98 (±16.92)	0.75 (±0.14)	-	1.87 (±0.43)	1.79 (±0.27)	0.97	assumption i
$\boldsymbol{y = a \cdot (kg)^{0.83} + 1/k_P \cdot x + 1/k_L \cdot z}$	**52.68 (±21.32)**	**0.83 (±0.15)**	**-**	**2.07 (±0.44)**	**1.66 (±0.27)**	**0.97**	assumption i
$y = a \cdot (kg)^b + 1/k_P \cdot x + 1/k_L \cdot z$	30.74 (±19.04)	0.79 (±0.22)	-	2.32 (±0.43)	1.92 (±0.26)	0.97	assumption ii
$y = a \cdot (kg)^{0.83} + 1/k_P \cdot x + 1/k_L \cdot z$	34.15 (±21.07)	0.83 (±0.22)	-	2.38 (±0.43)	1.88 (±0.26)	0.97	assumption ii

Tables 28 and 29 compare alternative calculations of the simple linear (DE intake versus energy gain) and multiple regression analyses (DE intake versus protein energy and lipid energy gain). According to the choice of calculation, slightly different values were obtained.

6.5 Discussion

6.5.1 Feed intake in relation to DCP/DE ratios

Digestible energy content is thought to be one of the major criteria controlling feed intake in fish (Lee and Putnam 1972; Jobling and Wandsvik 1983; Kentouri *et al*. 1995; Paspatis and Boujard 1996) along with other factors including fish size, temperature or palatability. In the present study gilthead seabream regulated voluntary feed intake according to DE rather than to dietary DCP which is apparent from Figure 9. Voluntary feed intake followed a bell shaped curve, where feed intake per g BW $(kg)^{-0.70}$ decreased with high dietary DE content (equation 24). Thus the total amount of DE consumed reached a plateau of maximum intake of about 282 kJ $kg^{-0.83}$ day^{-1}

(Figure 11a, equation 25). On the other hand, feed intake in gilthead seabream was reduced at low dietary DE densities as well. However, since low DE diets (Trial C, diets C1 - C6) were formulated with up to 36% cellulose, the possibility of palatability problems cannot be excluded. According to Bromley and Adkins (1984), trout could regulate their feed intake to make up for low energy density until 30% cellulose inclusion was reached when intake dropped drastically. This fact, together with an increase in stomach weight in trout fed a 40-50% cellulose diet, was interpreted by the authors, that the fish could not compensate by further increasing feed intake because of limiting stomach capacity. Feeding was also reduced in channel catfish, when given a diet with higher fibre levels, indicating that the extra bulk depressed the feed intake (Page and Andrews 1973).

In this study gilthead seabream was able, within limits, to compensate for a low energy feed by enhancing intake as far as the physical capacity of the digestive tract permitted. However, the feed intake indirectly influenced the protein consumption as well as seen in Figure 11a. Reduced intake of high energy diets on one hand, and at the other extreme, very low energy diets, decreased protein intake and thus caused growth depression (Figure 11b). A similar process was observed in trout, where caloric intake was regulated by feed, but protein intake was not compensated for by feed intake (Boujard and Médale 1994). Ellis and Reigh (1991) also suggested in their study with red drum, that low protein intake in combination with high dietary GE / CP ratios was growth-limiting.

6.5.2 Body composition in relation to DCP/DE ratio

The use of protein as an energy source is wasteful from an economical and ecological point of view, and more and more research has been done replacing protein as an energy source with lipid or carbohydrates. However, very often the level of lipid, or better the low level of protein may lead to an undesirable high lipid deposition in the fish carcass. As stated previously in chapter 3, the protein concentration in the body mass of seabream remained constant at 177g kg^{-1} whereas the lipid level increased with increasing fish size, when feeding the commercial diet. When seabream were fed diets with varying energy to protein in the trials C to D, the same pattern could be observed, at least concerning protein. Again whole body composition displayed narrow limits for protein, between 159 and 190g kg^{-1} but wide ranges for lipid content from 65 to 166g kg^{-1} weight (Figure 11c). This pattern occurred regardless of fish size suggesting additional dietary influences. Linear regression between fish size and fat deposition could account for 75% of the variation and regression between dietary DE and lipid retention could explain 93% of the variance.

Figure 17 illustrates the effect of varying DCP/DE ratios on the amount of lipid deposition in seabream in comparison to fish fed the “standard” commercial diet.

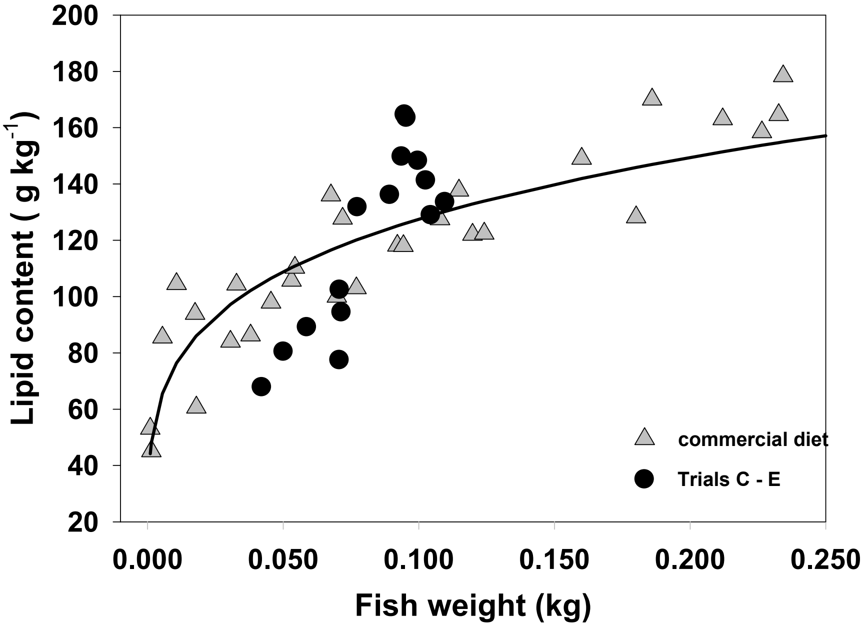

***Figure 17**: Lipid content of gilthead seabream fed diets with different DCP/DE ratios in comparison to the lipid content of seabream fed the commercial diet (according to Figure 3 and equation 9).*

There seems to be a common pattern where fish tend to increase their lipid deposition with increasing fat levels in diets in conjunction with decreasing protein intake. Feeding trout *ad libitum* with a non-protein energy source and restricted protein led to increasing levels of fat in the carcass (Kaushik and Luquet 1984). Under protein restriction, carp accumulated proportionally more dry matter, fat and energy (Schwarz *et al.* 1985). Similarly the dressing out percentage of Atlantic salmon dropped, and the fat in the filet was higher as the protein content of the diets decreased to 350g kg^{-1}, which corresponded to a DCP/DE ratio of 14.8 (Hillestad and Johnson 1994).

6.5.3 Energy and protein efficiency in relation to DCP/DE ratio

When feeding seabream of different sizes graded levels of DE (Trial A and B) the respective retention resulted in linear responses (equations 14 and 15). The differences between the slopes of the lines for the two fish sizes were small (0.49 and 0.54), but between the intercepts significant differences were observed (-1.81 and -4.38). This is expected, as the intercept represents the energy loss at starvation per fish. By using the metabolic body weight of $(kg)^{0.83}$, one can combine all fish weights to examine the relationship between DE and the respective retention (Figure 7). Furthermore, combining all the data sets of Trials A through E confirms the linearity between DE intake and energy retained (Figure 14) regardless of the DCP/DE ratio. Assuming linearity over the whole range studied as proposed in assumption i, the value of the intercept (equation 32) is somewhat different from the results of the starvation trial. Basing the regression on assumption ii however, results of intercepts for the equations describing the section of feeding levels 0 and 1 are very close to losses of energy observed in the starvation trial. This would indicate that assumption ii maybe biologically more justified. Using this scenario the maintenance requirement for energy in seabream was found to be 53.43 kJ DE $BW(kg)^{-0.83}$ day^{-1} and the efficiency of DE for maintenance was calculated as 0.76 (equation 33).

When multiple regression was employed, which estimated the maintenance requirement simultaneously, the constant term of the equation corresponded with the experimentally derived maintenance requirement. By combining all the feeding trials, A through E, the maintenance requirement was determined as 52.68 kJ DE $BW(kg)^{-0.83}$ day^{-1} (Table 29). Thus using different approaches a maintenance requirement of 53.0 kJ DE $BW(kg)^{-0.83}$ day^{-1} can be quantified for gilthead seabream. In a study by Huismann (1976) the maintenance requirement was 66 kJ ME $BW(kg)^{-0.80}$ day^{-1} and 48 kJ ME $BW(kg)^{-0.80}$ day^{-1} for carp and rainbow trout respectively and the partial efficiency of utilisation of ME for maintenance was 0.70 for both fish species. Meyer-Burgdorff *et al.* (1989) determined a similar maintenance requirement (57 kJ $(kg)^{-0.80}$ day^{-1}) for tilapia and Kirchgessner *et al.* (1984) estimated a DE_m of 45 kJ $(kg)^{-0.75}$ day^{-1} for carp. The similarities of maintenance requirement between the different fish species are not surprising as energy loss at starvation was also very similar.

The energy efficiency for growth (k_{DEg}) has been shown to be constant in seabream regardless of feed intake and DCP/DE ratio by different methods of calculation. The relationship between DE intake and energy gain is linear, however the values range from 0.49 ± 0.02 (equation 34) up to 0.56 (reciprocal of 1.78 ± 0.04, Table 28), according to the method of calculation. The fact, that energy efficiency is constant has been also demonstrated recently in rainbow trout where the utilisation of DE for gain (k_{DEg}) was 0.61 regardless of feeding level as well as temperature (Azevedo *et al*. 1998). This value is close to the k_{DEg} = 0.68 of another study with rainbow trout (Rodehutscord and Pfeffer 1999). The efficiency of utilisation for growth above maintenance, if assuming a value of k_{DEg} = 0.56, however, appears to be slightly lower in seabream in comparison to the other fish species.

In contrast to the linearity of the relationship between DE intake and energy retained, the DCP intake and protein retained using diets with varying protein content (Trials C to E) was better described by an exponential curve (Figure 13, equation 31). This pattern was confirmed by

combining the data of all the trials A through E, including the zero treatments (Figure 15, equation 35). At low DCP intake, the slope of the curve is the steepest, the response to increasing protein is the highest, thus the protein gain is at its most efficient - since it is limiting. The curve plateaus at a maximum daily protein gain, which was 1.89g protein $BW(kg)^{-0.70}$ (equation 35). Since protein gain reaches a maximum and voluntary feed intake is regulated by DE requirements it is likely that surplus protein is used as an energy source. Also, if the diet is deficient in non-protein energy, protein will be used for energetic purposes rather than for protein synthesis, causing reduced growth even with high dietary protein content hence resulting in lower efficiency. The differences in the protein response result from its utilisation both as a protein and as an energy source.
This is illustrated in Figure 16b, where the values of partial efficiencies of DCP for growth k_{DCPg} (Table 27) are shown in relation to DCP/DE ratios. At high dietary DCP/DE ratios we find that the efficiency of protein utilisation reaches a plateau around 0.37. A further increase of the DE content by raising the non-protein energy fraction improved the protein efficiency which reached k_{DCPg} = 0.56 at low DCP intake.
However, highest protein efficiency did not concur with maximum growth (Figure 16b), and it indicates limiting protein supply, as the following example illustrates. Maximum weight gain in gilthead seabream (Figure 16a; equation 39) could be achieved by providing a DCP/DE ratio of 19.1 for a 50g fish. On the other hand, to reach a maximum protein gain (Figure 16a; equation 38) corresponded to a DCP/DE ratio of 21.9. At DCP/DE ratios of 19.1 and 21.9 k_{DCPg} values of 0.48 and 0.43 respectively can be found (Figure 16b, equation 37). The difference in this response is due to the change in the relative composition of the weight gain (Figure 11c) as mentioned before. The value of k_{DCPg} = 0.43 in this case therefore characterises protein efficiency for maximum protein gain, higher protein efficiencies seem to be reached only at reduced overall gain. However both values can only be determined with a high degree of error.
These findings, where maximum growth and highest protein efficiency are not identical agrees with reports in other fish species such as tilapia (Kaushik *et al.* 1995) and trout (Kim and Kaushik 1992). In other studies with gilthead seabream, Vergara *et al.* (1996a) described decreasing PER values with increasing dietary protein levels from 420 to 580 g kg^{-1}, but weight gain was improved with the higher protein diets for gilthead seabream sized 5 to 30g. The same pattern was observed by Santinha *et al.* (1996), where a 55% protein diet gave the best growth in gilthead seabream, between 9 to 63g, but a 40% protein diet showed the highest protein efficiency.
Another point worth mentioning is, since protein requirement is in reality the sum of the amino acid requirements, k_{DCPg} is influenced by the protein sources used in this study with gilthead seabream. As most of the diets were formulated with fish meal where the amino acid composition is apparently balanced, the values of k_{DCPg} can be applied only to similar diets.

To further partition the energy retention into protein and lipid retention, and to estimate the efficiency for protein deposition, multiple regression was employed. Applying different mathematical models (Table 28 and 29) partial efficiencies for protein k_P were found to range between 0.42 - 0.53 and efficiencies for lipid k_L between 0.52 - 0.60. In this case, the values of k_{DCPg} (0.43 - 0.48) which are defined as the efficiency of utilisation of DCP for growth are nearly identical to the values of k_P which describe the energy cost to deposit protein.

Using a similar mathematical approach, Meyer-Burgdorff and Rosenow (1995) reported values for common carp ranging between k_P = 0.56 - 0.58 and k_L = 0.79 - 0.86 for protein and lipid, respectively, and values of k_P = 0.56 and k_L = 0.72 have been reported by Schwarz and Kirchgessner (1995) for common carp as well. The studies done with other fish species show on average higher efficiencies for lipid utilisation than for protein. Overall efficiency in seabream was estimated as k_{DEg} = 0.56 and as both protein and lipid energy contribute, an average k_P of 0.48 and k_L of 0.60 can be assumed.

It is well known from higher animals, that the synthesis of protein is less efficient than the synthesis of lipids. In warm-blooded monogastric animals, a narrow range of regression coefficients have been found for lipid depositions and a much wider range for protein deposition (Klein and Hoffmann 1989) and were on average k_P = 0.55 and k_L = 0.81. There is also a higher discrepancy between the theoretically derived efficiency for protein synthesis k_P = 0.87 compared to the experimentally derived efficiency of only 0.48 (Buttery and Boorman 1976). Obviously protein deposition includes the sum of synthesis and catabolism as well as other energy consuming processes such as amino-acid transport, formation and excretion of ammonia (in fish). Meyer-Burgdorff and Rosenow (1995) suggest that in growing common carp the protein turnover, exceeding protein synthesis is the main reason for a rather low energy efficiency for protein deposition.

In contrast, the efficiency of lipid retention agrees well with the value calculated stoichiometrically. Pullar and Webster (1977) found values of 0.44 and 0.73 in rat for protein and lipid efficiency, respectively. Emmans (1994) calculated in pigs a lipid retention efficiency of k_L = 0.90 if lipid was formed from dietary lipid and k_L = 0.71 if lipid was formed from non-lipids such as carbohydrates. For gilthead seabream a low value of k_L = 0.60 was found, suggesting that in addition carbohydrate as well as protein energy was involved in lipid deposition, however overall energy efficiency was found to be lower compared to other fish species, which would be reflected of course in the k_P and k_L values.

7 Implications for optimal feeding

The results of this study taken together allow calculation of the daily recommended intake for growing gilthead seabream. The derived parameters of energy and protein demand can be used to develop models that dictate the required dietary composition, at least in protein and energy terms, for gilthead seabream at any phase of its life cycle. By defining the fish's demands for maintenance and growth a comprehensive energy budget can be derived that essentially quantifies the energy the fish needs to consume to achieve its growth potential at any specific temperature and part of its growth cycle (Table 30).

Table 30: *Recommendations for supply of DE to gilthead seabream at two different growth rates (at 20 and 26°C).*

Body weight, g fish^{-1}	*10*		*50*		*100*		*350*	
Metabolic BW, kg$^{0.83}$	0.022		0.083		0.148		0.418	
DE_m[1], kJ fish^{-1} day^{-1}	1.15		4.38		7.79		22.04	
Water temperature	*20°C*	*26°C*	*20°C*	*26°C*	*20°C*	*26°C*	*20°C*	*26°C*
Weight gain[2], g day^{-1}	0.21	0.31	0.55	0.81	0.83	1.23	1.76	2.60
RE[3], kJ fish^{-1} day^{-1}	1.39	2.06	4.43	6.54	7.29	10.77	17.95	26.51
DE_g[4], kJ fish^{-1} day^{-1}	2.48	3.66	7.89	11.64	12.98	19.17	31.95	47.19
DE_{m+g}[5], kJ fish^{-1} day^{-1}	3.63	4.81	12.27	16.03	20.77	26.96	53.99	69.23
% of total DE used for maintenance	31.7	23.9	34.8	27.3	37.5	28.9	40.8	31.8

[1] Digestible energy required for maintenance = 53.0 kJ kg$^{-0.83}$ day^{-1}
[2] Predicted weight gain for gilthead seabream at 20 and 26°C respectively, equation (2)
[3] Retained energy = body energy • weight gain (equation 6)
[4] Digestible energy required for growth - using efficiency for growth k_{DEg} = 0.56
[5] Total DE required for maintenance and growth

Comparison of the maintenance energy demands with those required for growth show that the influence of body composition on energy demand is greater than that of requirements for maintenance metabolism. This is particularly so when the fish is small and its growth rate far higher on a relative basis. For example at 26°C the energy demands of a 10g seabream are 24% for maintenance and 76% for growth, whereas for a 350g seabream energy demands are 32% for maintenance and 68% for growth. So it is obvious, that the proportion of total DE which is required for maintenance will increase with increasing body weight and with decreasing growth rate. The influence of temperature on energy demands by gilthead seabream for maintenance were only briefly explored in this study, as the temperature range was apparently too narrow. The nature of this energetic response to temperature needs to be more fully explored to define critical limits to energy use efficiency and how these may change under varying temperature regimes. To further adapt those feeding regimes to the Mediterranean Sea as the temperature in this area covers a range from 13 up to 29°C.

The absolute protein requirement per day per fish is dependent on fish size and weight gain, regardless of dietary DE density. Therefore, the protein level expressed as a percentage of the feed will change according to the preferred DE level (Table 31). The DCP/DE ratio will decrease with increasing fish size and decreasing growth potential, as demonstrated in Table 31, due to the changing ratio of energy to protein of the gain and the increasing proportion of energy used for maintenance with increasing fish size.

This has been demonstrated in the study of García-Alcázar *et al.* (1994) for gilthead seabream, where smaller fish up to 100g grow better on a 49% protein, 12% lipid diet while bigger fish up to 330g performed better on a 45% protein, 19% lipid diet. The optimal DCP/DE ratio also decreased in Atlantic salmon with increasing fish weight (Einen and Roem 1997).

Current assessments of protein requirements for trout are 22 - 24 g MJ^{-1} DCP/DE (Cho and Kaushik 1990; Cho 1992) and for carp 20 - 22 g MJ^{-1} CP /DE (Schwarz *et al*. 1983, Zeitler *et al.* 1984). These values of course would depend on fish size, growth rate and composition of the gain as calculated in Table 31.

Fish, that are able to consume high amounts of feed due to increased stomach capacity, could be fed lower energy diets with low protein levels, since, based on the calculation from Table 31, the same amount of protein per day would be consumed. It has been reported that the optimum dietary protein content changes in carp and rainbow trout with increasing feeding intensity (Ogino 1980): in carp the optimal crude protein content decreased from 50% to 35% with an increasing feed intake from 2.5 to 3.5% . Beamish and Medland (1986) found, that growth rate in large trout had significantly improved on a high protein diet, but the effects were only apparent at restricted feeding.

__Table 31__: Recommendations of dietary energy and protein supply for growing gilthead seabream (at 23^{0}C), when formulating feeds with different energy contents.

Body weight, g fish^{-1}	*10*		*50*		*100*		*350*	
Weight gain[1], g day^{-1}	0.26		0.67		1.01		2.14	
Energy requirement								
Metabolic BW, kg$^{0.83}$	0.022		0.083		0.148		0.418	
DE_m[2], kJ fish^{-1} day^{-1}	1.15		4.38		7.79		22.04	
DE_g[2], kJ fish^{-1} day^{-1}	3.01		9.58		15.77		38.83	
DE_{m+g}[2], kJ fish^{-1} day^{-1}	4.16		13.97		23.56		60.87	
Protein requirement								
Metabolic BW, kg$^{0.70}$	0.040		0.123		0.200		0.480	
DCP_m[3] ,g fish^{-1} day^{-1}	0.031		0.095		0.154		0.369	
RCP [4], g fish^{-1} day^{-1}	0.045		0.118		0.179		0.378	
DCP_g[5], g fish^{-1} day^{-1}	0.094		0.245		0.370		0.783	
DCP_{m+g}[6], g fish^{-1} day^{-1}	0.125		0.340		0.524		1.152	
Feed formulation								
DE density of feed MJ kg^{-1}	*15 MJ*	*19 MJ*	*15 MJ*	*19 MJ*	*15 MJ*	*19 MJ*	*15 MJ*	*19 MJ*
Feed intake, g fish^{-1} day^{-1}	0.28	0.22	0.93	0.74	1.57	1.24	4.06	3.20
DCP content in feed, g kg^{-1}	448	568	365	462	338	423	284	360
FCR	1.09	0.86	1.39	1.10	1.55	1.23	1.90	1.50
DCP/DE ratio g MJ^{-1}	29.9	29.9	24.3	24.3	22.2	22.2	18.9	18.9

[1] Predicted weight gain for gilthead seabream at 23°C, equation (2)
[2] values see Table 19.
[3] Digestible crude protein required for maintenance - 0.77 g kg$^{-0.70}$ day^{-1}
[4] Retained protein = body protein (177g kg^{-1}) ·weight gain
[5] Digestible crude protein required for growth - using $k_{DCPg} = 0.48$
[6] Total DCP required for maintenance and growth

Due to the fact that protein and energy demands are constantly changing, different diets would have to be formulated for growing gilthead seabream. However, on a practical basis it is unreasonable to expect that a large range of diets would be used to support production of any fish species (Table 32).

Table 32*: Proposed diet formulation (kg $^{-1}$) and corresponding practical feeding table for gilthead seabream during the whole growing period (at 23 °C).*

Weight (g)	Feed composition	Weight gain g fish^{-1} day^{-1}	Feed intake g fish^{-1} day^{-1}	Days of growth	FCR
1	540g CP*, 19.8 MJ GE*	0.06	0.06		1.00
5	520g CP, 19.8 MJ GE	0.16	0.17	37	1.09
10	480g CP, 20.0 MJ GE	0.24	0.29	62	1.20
50	450g CP, 20.7 MJ GE	0.66	0.88	153	1.37
100	420g CP, 21.0 MJ GE	1.00	1.47	214	1.47
200	400g CP, 21.5 MJ GE	1.53	2.38	293	1.60
350	400g CP, 22.0 MJ GE	2.16	3.60	374	1.67

* assuming ADC for protein of 85% and for energy of 80%

In Table 32 a range of diets specifically suited to the indicated production periods have been suggested, though these remain to be tested under practical conditions. Effectively what this indicates is that at smaller fish sizes it is more practical to use lower energy diets, while above 200g gilthead seabream production would be better served by using a diet with a GE content of 22 MJ kg^{-1} or greater, which can be only achieved by high lipid levels. This estimation process also allows some accommodation for changing dietary intake capacities. Notably, the upper and lower extremes of this interpretation have little practical basis, with it being difficult to create a 19 MJ DE, 568g DP kg^{-1} diet to feed to smaller fish (Table 31) and similarly the amount of feed consumption required of a 15 MJ DE diet by a 350g fish is approaching the physical limits of gilthead seabream (equation 5).

Conclusions

This study demonstrates the use of he factorial approach to determine the requirements for DE and DP and the interactions between them in gilthead seabream. The energy maintenance requirement is 53.0 kJ kg$^{-0.83}$ day^{-1} and efficiency for growth is $k_{DEg} = 0.56$. The optimal protein requirements have been determined and optimal ratios at different dietary energy in the feed estimated by two different approaches.

These data have been used to formulate feeding tables for farming of gilthead seabream which are now applied in culture. Using this model, it is possible to calculate the biological and economical efficiency of different diets and optimise the feeding regime. Another benefit is that nutrient budget calculations can be carried out for different feeds. In open net cages the models developed here can be used to estimate their impact on the eutrophication of the water and will assist in decision making of the relevant authorities.

8 Attachment

Table A 1*: Data set of weight, weight gain and feed intake at different fish weights and water temperatures collected from various experiments with gilthead seabream fed a commercial diet.*

Days of growth	Weight g fish^{-1}	Mean weight g fish^{-1}	Weight gain g fish^{-1} day^{-1}	Feed intake g fish^{-1} day^{-1}	Temperature ^{0}C
0	0.72				
13	1.25	0.95	0.04	0.05	23.9
27	1.95	1.56	0.05	0.06	23.1
45	3.22	2.51	0.07	0.09	21.6
61	5.55	4.23	0.15	0.18	21.5
76	7.99	6.66	0.16	0.25	21.8
90	11.17	9.45	0.23	0.34	20.7
112	18.85	14.51	0.35	0.56	20.9
127	24.47	21.48	0.37	0.49	20.8
0	19.14				
19	31.62	24.60	0.66	1.06	25.0
28	36.28	33.87	0.52	0.82	24.4
43	47.02	41.30	0.72	1.15	24.0
64	64.08	54.89	0.81	1.31	23.8
81	78.56	70.95	0.85	1.39	23.0
0	27.33				
14	37.55	32.04	0.73	0.89	21.9
28	46.20	41.65	0.62	0.71	22.8
42	56.11	50.91	0.71	0.82	23.0
56	66.56	61.11	0.75	1.03	23.2
72	78.06	72.08	0.72	0.94	23.3
93	97.87	87.41	0.94	1.13	24.1
0	2.78				
13	5.88	4.04	0.24	0.30	26.1
28	11.42	8.19	0.37	0.49	26.1
45	20.59	15.33	0.54	0.67	25.5
57	28.38	24.17	0.65	0.96	25.5
92	51.87	38.37	0.67	0.98	23.9

Table A1 continued

Days of growth	Weight g fish^{-1}	Mean weight g fish^{-1}	Weight gain g fish^{-1} day^{-1}	Feed intake g fish^{-1} day^{-1}	Temperature ^{0}C
0	33.35				
19	46.44	39.35	0.69	0.73	22.0
34	57.98	51.89	0.77	0.99	22.8
47	71.01	64.17	1.00	1.22	23.0
62	84.68	77.54	0.91	1.22	23.2
75	99.87	91.96	1.17	1.48	23.3
91	116.00	107.63	1.01	1.59	24.1
0	17.52				
14	22.41	19.81	0.35	0.36	20.9
28	26.42	24.33	0.29	0.38	21.0
45	33.20	29.62	0.40	0.50	21.0
59	39.15	36.05	0.43	0.61	21.8
73	45.16	42.05	0.43	0.59	21.9
87	52.26	48.58	0.51	0.71	22.5
101	62.46	57.13	0.73	1.01	22.9
118	76.95	69.33	0.85	1.22	23.2
0	1.02				
14	1.70	1.32	0.05	0.06	20.7
28	2.55	2.08	0.06	0.07	20.9
42	3.91	3.16	0.10	0.11	20.8
56	5.70	4.72	0.13	0.15	21.0
73	8.13	6.81	0.14	0.17	20.9
86	10.52	9.25	0.19	0.23	21.2
103	14.96	12.55	0.26	0.34	21.5
115	18.79	16.77	0.32	0.40	22.0
131	24.79	21.58	0.38	0.45	22.7
0	2.20				
16	4.04	2.98	0.12	0.18	26.0
30	5.96	4.91	0.14	0.23	25.3
60	14.66	9.35	0.29	0.39	25.0
72	18.68	16.55	0.34	0.53	24.4
84	24.75	21.50	0.51	0.59	24.3
96	31.35	27.86	0.55	0.79	24.0

Table A1 continued

Days of growth	Weight g fish^{-1}	Mean weight g fish^{-1}	Weight gain g fish^{-1} day^{-1}	Feed intake g fish^{-1} day^{-1}	Temperature ^{0}C
0	2.34				
13	3.66	2.93	0.10	0.14	22.0
25	4.98	4.27	0.11	0.15	21.8
40	7.13	5.96	0.14	0.21	21.8
58	12.22	9.33	0.28	0.39	20.9
71	16.06	14.01	0.30	0.43	20.2
83	21.55	18.60	0.46	0.68	20.9
101	32.05	26.28	0.58	0.90	21.8
109	36.65	34.27	0.58	0.82	22.0
0	76.29				
21	94.37	84.85	0.86	1.69	26.0
36	109.15	101.49	0.99	1.51	25.4
50	124.98	116.80	1.13	1.67	24.8
64	143.05	133.71	1.29	1.86	24.1
74	156.90	149.82	1.39	2.29	24.2
107	209.50	181.30	1.59	2.37	24.0
124	234.70	221.74	1.48	2.51	23.5
135	254.80	244.54	1.83	3.10	22.5
162	305.10	278.82	1.86	3.60	21.8
182	342.56	323.29	1.87	3.54	22.0
0	197.00				
29	239.10	217.03	1.45	2.87	22.9
38	248.12	243.57	1.00	2.18	23.3
75	316.02	280.02	1.84	3.60	24.1
101	354.60	334.75	1.48	2.93	25.2
132	424.70	388.07	2.26	4.70	26.0
0	64.43				
11	74.41	69.24	0.91	1.29	26.0
32	98.96	85.81	1.17	1.71	25.5
41	111.69	105.13	1.41	1.97	25.6
94	187.54	144.73	1.43	2.19	25.0
107	204.10	195.64	1.27	2.00	25.2
129	239.90	221.28	1.63	2.51	24.5
147	273.50	256.15	1.87	3.12	23.0
172	314.60	293.33	1.64	2.84	22.0
198	360.90	336.96	1.78	3.98	22.6
229	418.34	388.56	1.85	3.35	20.0
267	477.70	447.04	1.56	3.01	21.5

Table A1 continued

Days of growth	Weight g $fish^{-1}$	Mean weight g $fish^{-1}$	Weight gain g $fish^{-1}$ day^{-1}	Feed intake g $fish^{-1}$ day^{-1}	Temperature ^{0}C
0	193.57				
23	235.15	213.35	1.81	2.93	24.8
43	284.12	258.48	2.45	3.92	24.2
60	328.01	305.28	2.58	4.21	24.0
78	378.20	352.21	2.79	4.48	23.2
100	418.00	397.60	1.81	4.23	23.0

***Table A 2**: Weight and body composition (kg^{-1} WW) of gilthead seabream before and after starvation*

Before starvation						*After starvation*					
Weight g fish^{-1}	Moisture g	Protein g	Lipid g	Ash g	Energy MJ	Weight g fish^{-1}	Moisture g	Protein g	Lipid g	Ash g	Energy MJ
0.98	754	148	53	37	5.40						
1.42	770	147	45	41	4.99	1.15	800	135	22	49	3.76
5.49	713	173	86	52	7.23	4.73	735	160	85	44	6.30
10.70	690	162	105	36	7.25	8.47	740	146	57	66	5.95
17.51	692	168	94	45	7.33	14.45	719	156	75	54	6.28
30.60	695	174	84	46	7.06	25.30	740	157	48	60	5.02
32.80	655	199	104	53	7.91	26.30	738	156	43	67	5.30
37.97	696	177	86	45	6.89	31.06	741	161	52	56	5.45
45.50	684	171	98	48	7.54	40.31	701	168	70	53	6.74
53.10	676	184	106	48	7.84	45.50	709	165	83	47	6.60
54.30	677	170	110	42	8.16	48.30	717	163	76	48	6.48
67.60	636	173	136	43	7.95	59.20	719	177	46	54	7.27
69.80	679	185	100	44	7.55	56.10	722	175	57	57	5.75
71.90	643	185	128	44	8.93	63.55	687	172	103	48	7.42
92.10	666	178	118	43	8.88	81.50	703	162	89	49	7.43
94.30	638	197	118	45	8.83	80.10	673	186	91	55	7.48
107.80	658	183	128	42	8.84	95.80	676	185	108	50	7.54
114.80	644	185	138	45	8.88	104.70	676	170	102	46	7.77
119.80	676	172	122	36	8.41						
124.10	660	176	122	49	8.05	107.40	670	182	100	54	7.34
160.00	629	184	149	46	9.41	134.60	673	161	118	57	7.72
180.10	651	187	128	43	8.74	160.68	683	184	97	46	7.47
186.00	605	171	170	38	10.09						
212.00	620	170	163	38	9.82						
226.50	610	190	158	50	9.93	207.70	635	191	137	51	8.98
232.70	626	181	165	42	10.08	206.40	668	177	99	47	9.96
234.30	591	188	178	43	10.38	194.30	624	196	145	52	9.41
284.00	610	166	170	37	9.97						
291.00	604	175	173	40	10.29						
310.80	594	180	177	35	10.55						
331.00	628	162	172	42	9.95						
364.40	584	190	185	33	10.86						
421.60	581	186	183	45	10.93						

Table A 3: *Energy (kJ) and protein (g) loss at starvation for gilthead seabream of different sizes.*

			Energy			*Protein*		
Mean Weight g fish^{-1}	Days	Temp ^{0}C	Initial kJ fish^{-1}	Final kJ fish^{-1}	Loss kJ fish^{-1} day^{-1}	Initial g fish^{-1}	Final g fish^{-1}	Loss g fish^{-1} day^{-1}
1.28	16	20.6	7.1	4.3	0.17	0.21	0.16	0.003
5.10	18	21.0	39.7	29.8	0.55	0.95	0.76	0.011
9.51	26	23.2	77.4	50.4	1.04	1.73	1.24	0.019
15.91	34	24.5	128.3	90.7	1.11	2.95	2.25	0.021
27.82	42	23.5	216.0	127.0	2.12	5.32	3.97	0.032
29.37	53	25.4	259.4	139.4	2.26	6.51	4.09	0.046
34.34	35	23.0	261.6	169.3	2.64	6.72	5.00	0.049
42.80	34	24.1	342.5	271.7	2.08	7.76	6.79	0.029
49.15	26	23.2	416.3	300.2	4.47	9.75	7.52	0.086
51.21	29	20.9	443.2	313.0	4.49	9.24	7.87	0.047
63.26	25	23.0	537.4	430.2	4.29	11.70	10.46	0.049
62.59	53	25.9	527.1	322.4	3.86	12.93	9.84	0.058
67.59	44	20.7	641.6	471.4	3.87	13.28	10.93	0.053
86.63	36	23.5	817.7	605.4	5.90	16.40	13.20	0.089
86.91	35	23.8	832.5	599.5	6.66	18.59	14.91	0.105
101.62	36	20.3	952.6	722.6	6.39	19.77	17.76	0.056
109.63	28	22.4	1019.7	813.9	7.35	21.19	17.81	0.121
115.45	40	24.8	999.5	788.1	5.28	21.87	19.57	0.058
146.75	53	25.8	1505.0	1039.0	8.79	29.50	21.74	0.147
170.08	36	20.5	1573.1	1199.7	10.37	33.69	29.61	0.113
216.90	40	23.9	2249.9	1864.1	9.65	43.04	39.67	0.084
219.16	25	23.0	2345.6	2056.3	11.57	42.19	36.57	0.225
213.36	42	23.7	2539.2	1829.2	16.91	44.05	38.12	0.141

***Table A 4**: Composition of feeds and faecal matter (kg^{-1} DM) and estimated ADC values (%) for protein rich single ingredients.*

	CP g	CL g	OM g	Heat of combustion MJ	Chromium g
Fish meal A	740	95	861	21.72	5.80
Faecal matter	339	12.5	477	9.98	23.36
	362	19.8	457	9.62	24.07
Fish meal B	618	87	858	21.07	6.02
Faecal matter	286	18.3	597	10.55	23.81
	283	17.2	577	10.00	24.05
Fish meal C	685	135	869	22.35	5.47
Faecal matter	375	14	652	12.62	15.81
	349	16.6	634	14.45	17.78
	364	26.4	631	14.35	16.94
Meat meal	626	122	826	20.40	5.22
Faecal matter	287	33.5	503	9.35	11.09
	275	29.5	509	9.15	10.81
Poultry meal	670	152	843	21.65	5.34
Faecal matter	305	28.4	482	10.26	11.77
	307	18.0	428	9.92	12.39
Squid meal	744	99.0	915	23.56	5.68
Faecal matter	402	83.0	756		21.35
	374	57.0	733	14.95	23.54
	377	62.0	755		26.28

***Table A 5**: Composition of feeds (test + reference ingredient), faecal matter (kg^{-1} DM) and resulting ADC (%) values using equation (12).*

	CP g	CL g	CC g	OM g	Heat of combustion MJ	Chromium g
Blood meal	766	79.0		893	22.60	5.54
Faecal matter	341	20.7		621		17.72
	335	29.1		618	13.08	17.31
	299	17.4		580		16.49
Soybean 250	635	106.0	132	873	20.90	5.34
Faecal matter	288	20.5	394	703	12.80	14.85
Soybean 750	530	56.0	319	905	20.10	5.75
Faecal matter	163	7.0	597	767	12.52	11.78
Soybean + Fish oil	434	72.0	422	928	21.00	5.13
Faecal matter	117	8.0	648	773	13.33	10.54

***Table A 6**: Composition of feeds (test + reference ingredient), faecal matter (kg^{-1} DM) and ADC values (%) for whole diets containing two levels of carbohydrate rich ingredients.*

	CP g	CL g	CC g	OM g	Heat of combustion MJ	Chromium g
Fish meal D	687	129		841	21.06	6.23
Faecal matter	328	31		517	11.50	15.78
	338	30		511	12.05	14.32
Soybean 20%	628	112	116	856	21.41	6.09
Faecal matter	263	33	278	574	13.55	14.85
	256	31	294	581	12.60	13.75
Soybean 40%	534	56	307	897	20.23	5.86
Faecal matter	164	30	519	713	12.46	12.24
	151	28	514	693	12.16	10.78
Cornmeal 20%	577	121	163	861	20.96	6.09
Faecal matter	288	22	290	600	11.16	14.03
	274	23	308	605	11.65	14.23
Cornmeal 40%	452	95	343	890	19.83	6.30
Faecal matter	210	20	424	654	13.73	12.18
	226	21	381	628	13.46	14.37
Cornstarch 20%	558	110	193	861	20.89	6.30
Faecal matter	313	22	197	532	11.87	17.83
	322	17	191	530	11.85	17.11
Cornstarch 40%	435	88	368	891	20.02	6.43
Faecal matter	269	15	281	572	12.20	18.17
	257	20	284	582	11.62	16.71
Wheat meal 20%	580	118	163	861	20.23	6.48
Faecal matter	303	24	252	579	12.46	15.74
	301	25	219	545	12.44	15.05
Wheat meal 40%	481	92	313	886	20.13	6.19
Faecal matter	221	21	377	619	13.09	13.41
	228	19	320	567	12.16	14.64

Table A 7: *Apparent digestibility coefficients (%) for carbohydrate rich ingredients tested at two dietary inclusion levels.*

	CP	CL	CC	OM	GE
Blood meal					
ADC (%)	89.5			81.1	
	89.3			80.0	82.9
	91.1			80.9	
Fish oil					
ADC (%)		93.9			90.0
		96.1			101.8
Soybean 250					
ADC (%)	87.5	53.7	-7.3	57.8	71.2
Soybean 750					
ADC (%)	86.1	91.3	8.7	52.6	65.5
Soybean 900					
ADC (%)	86.9	93.1	25.3	56.5	65.2
Soybean 250					
ADC (%)	94.3	41.7	1.7	66.7	65.0
	89.2	37.5	-12.3	57.2	64.6
Soybean 750					
ADC (%)	87.8	37.4	19.1	58.1	68.1
	86.8	32.0	9.0	52.8	63.9
Cornmeal 200					
ADC (%)	43.7	119.0	22.8	52.7	77.3
	75.4	115.8	19.1	53.5	73.5
Cornmeal 400					
ADC (%)	43.6	83.3	36.1	46.0	43.5
	63.6	90.8	51.3	62.1	59.6
Cornstarch 200					
ADC (%)			63.9	90.1	95.2
			63.6	86.2	90.5
Cornstarch 400					
ADC (%)			73.0	80.6	81.4
			70.3	75.2	79.4
Wheat meal 200					
ADC (%)	51.1	129.2	36.4	64.4	65.0
	34.6	109.7	42.2	66.1	59.0
Wheat meal 400					
ADC (%)	70.8	82.5	44.4	59.2	58.8
	80.7	102.3	56.8	70.7	70.8

Table A 8: *Composition of feeds and faecal matter (kg^{-1} DM) and calculated ADC values for whole diets mixed from single ingredients to test additivity.*

	CP g	CL g	CC g	OM g	Heat of combustion MJ	Chromium g
Diet 1	511	138	236	885	21.72	5.82
Faecal matter	221	42	366	629	13.47	15.88
Diet 2	521	138	217	876	21.89	6.25
Faecal matter	179	41	420	640	13.19	15.99
Diet 3	508	146	231	885	21.63	6.44
Faecal matter	253	25	218	496	11.60	16.62
Diet 4	515	139	229	883	21.38	6.03
Faecal matter	237	27	186	450	11.28	22.57
Diet 5	516	149	220	885	21.20	6.23
Faecal matter	199	25	266	490	11.86	12.16

***Table A 9**: Predicted contents of DE and DCP (kg^{-1} DM) and ADCs (%) for whole diets formulated to test additivity.*

	Diet1	Diet2	Diet3	Diet4	Diet5
Formulation					
Fish meal C	350	350	320	300	140
Meat meal	200		200	180	130
Poultry meal			150		140
Blood meal		150		160	135
Soybean meal	220	220			160
Wheat meal	150	150	250	250	190
Fish oil	50	80	50	70	70
Energy, MJ					
GE	21.20	21.77	21.36	21.83	21.63
DE	16.15	16.99	16.56	17.22	16.76
ADC %	76.18	78.01	77.56	78.89	77.50
Protein, g					
CP	500	506	487	503	500
DCP	413	432	394	420	418
ADC (%)	82.54	85.40	80.83	83.60	83.70
Lipid, g					
CL	131	139	147	143	138
DCL	119	128	137	133	126
ADC (%)	91.34	91.87	93.28	92.73	91.36
Organic Matter, g					
OM	875	883	868	879	883
DOM	607	632	633	653	632
ADC(%)	69.33	71.62	73.01	74.27	71.57
Carbohydrate, g					
CC	219	219	206	206	226
DCC	87	87	105	105	97
ADC (%)	39.68	39.68	50.97	50.97	43.02

Table A 10: *Composition of diet A (kg^{-1} DM) and predicted DE and DCP content.*

	Diet A
Formulation	
Fish meal A	885
Fish oil	95
Energy MJ kg^{-1}	
GE	22.86
DE	20.60
ADC(%)	90.1
Protein g kg^{-1}	
CP	675
DCP	594
ADC (%)	88.0

Table A 11*: Composition of commercial diet B and faecal matter (kg^{-1} DM) and ADCs (%) for energy and nutrients.*

	CP g	CL g	CC g	OM g	GE MJ	Chromium g
Diet B	394	93	291	903	20.28	8.81
Faecal matter	193	26	290	773	15.91	27.3
	173	25	325	760	15.02	28.5

***Table A 12**: Body composition (kg^{-1} WW) of gilthead seabream in Trials A and B.*

Treatment	Weight g fish^{-1}	Dry matter g	Protein g	Lipid g	Ash g	Energy MJ
Trial A						
Initial	30.08	305	174	84	46	7.06
zero	24.59	255	162	42	61	4.84
zero	25.28	274	161	61	63	5.61
zero	25.41	250	155	42	56	4.74
low	39.22	304	184	78	50	6.85
low	41.94	310	178	90	47	7.21
low	37.83	300	176	87	43	6.71
medium	53.22	322	179	101	43	7.45
medium	55.47	327	181	109	43	8.01
medium	55.72	322	177	109	43	7.64
high	67.06	364	190	136	43	9.18
high	65.06	340	173	130	40	8.45
high	63.18	345	173	126	44	8.48
Trial B						
Initial	92.00	344	178	118	43	8.76
zero	80.00	301	159	95	49	7.22
zero	81.00	308	164	107	49	7.73
zero	83.44	313	165	105	48	7.80
low	99.56	345	175	128	48	8.94
low	105.61	350	172	137	46	8.75
low	105.28	348	171	140	43	9.08
medium	118.89	348	171	143	41	9.09
medium	118.89	345	171	138	43	9.05
high	132.44	365	177	151	43	9.87
high	130.78	355	173	150	40	9.82
high	130.00	354	171	151	40	9.80

Table A 13: *Feed intake and retention of energy and protein in fish of Trial A after 42 days of growth.*

Trial A	*zero*	*zero*	*zero*	*low*	*low*	*low*	*medium*	*medium*	*medium*	*high*	*high*	*high*
Weight initial (g)	30.60	30.30	30.90	29.30	30.80	29.80	29.80	29.30	31.10	30.00	29.60	29.40
Weight final (g)	24.59	25.28	25.41	39.22	41.94	37.83	53.22	55.47	55.72	67.06	65.06	63.18
Average weight (g)	27.43	27.68	28.02	33.90	35.94	33.58	39.82	40.31	41.63	44.85	43.88	43.10
Weight gain (g $fish^{-1}$ day^{-1})	-0.143	-0.120	-0.131	0.236	0.265	0.191	0.558	0.623	0.586	0.882	0.844	0.804
Food fed (g $fish^{-1}$ day^{-1})				0.282	0.292	0.272	0.633	0.648	0.661	1.030	1.002	1.012
DE fed (kJ $fish^{-1}$ day^{-1})				5.92	6.13	5.70	13.27	13.58	13.87	21.59	21.01	21.22
DE fed (kJ $kg^{-0.83}$ day^{-1})				98.17	96.89	95.39	192.61	195.12	194.07	283.95	281.36	288.50
DP fed (g $fish^{-1}$ day^{-1})				0.168	0.174	0.162	0.376	0.385	0.394	0.613	0.596	0.602
DCP fed (g $kg^{-0.70}$ day^{-1})				1.79	1.78	1.74	3.59	3.65	3.64	5.38	5.32	5.44
Energy gain (kJ $fish^{-1}$ day^{-1})	-2.31	-1.72	-2.33	1.47	2.02	1.03	4.43	5.65	4.91	9.61	8.11	7.81
Energy gain (kJ $kg^{-0.83}$ day^{-1})	-45.70	-33.71	-45.22	24.42	31.97	17.30	64.32	81.25	68.68	126.46	108.67	106.24
Protein gain (g $fish^{-1}$ day^{-1})	-0.032	-0.029	-0.034	0.050	0.050	0.035	0.103	0.118	0.106	0.179	0.145	0.138
Protein gain (g $kg^{-0.70}$ day^{-1})	-0.40	-0.35	-0.42	0.54	0.51	0.38	0.99	1.11	0.98	1.57	1.30	1.25
Lipid gain (g $fish^{-1}$ day^{-1})	-0.037	-0.024	-0.036	0.014	0.028	0.019	0.068	0.085	0.082	0.157	0.142	0.131
FCE				0.84	0.91	0.70	0.88	0.96	0.89	0.86	0.84	0.79
Protein gain / DCP intake				0.30	0.29	0.22	0.27	0.31	0.27	0.29	0.24	0.23
Energy gain/ DE intake				0.25	0.33	0.18	0.33	0.42	0.35	0.45	0.39	0.37
metabolic weight $kg^{0.83}$	0.051	0.051	0.051	0.060	0.063	0.060	0.069	0.070	0.071	0.076	0.075	0.074
metabolic weight $kg^{0.70}$	0.081	0.081	0.082	0.094	0.097	0.093	0.105	0.106	0.108	0.114	0.112	0.111

Table A 14: Feed intake and retention of energy and protein in fish of Trial B after 36 days of growth.

	zero	zero	zero	low	low	low	medium	medium	high	high	high
Weight initial (g)	91.80	91.70	92.80	90.89	94.39	92.50	93.20	91.80	93.56	92.67	93.83
Weight final (g)	80.00	81.00	83.44	99.56	105.61	105.28	118.89	118.89	132.44	130.78	130.00
Average weight (g)	85.70	86.18	88.00	95.13	99.84	98.68	105.26	104.47	111.32	110.09	110.44
Weight gain (g $fish^{-1}$ day^{-1})	-0.328	-0.297	-0.260	0.241	0.312	0.355	0.714	0.753	1.080	1.059	1.005
Food fed (g $fish^{-1}$ day^{-1})				0.723	0.751	0.742	1.539	1.511	2.197	2.160	2.141
DE fed (kJ $fish^{-1}$ day^{-1})				10.92	11.34	11.20	23.24	22.82	33.17	32.61	32.33
DE fed (kJ $kg^{-0.83}$ day^{-1})				76.94	76.76	76.55	150.55	148.76	205.19	203.60	201.31
DP fed (g $fish^{-1}$ day^{-1})				0.242	0.252	0.248	0.516	0.506	0.736	0.724	0.717
DCP fed (g $kg^{-0.70}$ day^{-1})				1.26	1.26	1.26	2.49	2.46	3.42	3.39	3.35
Energy gain (kJ $fish^{-1}$ day^{-1})	-6.29	-4.92	-4.50	2.61	2.70	4.05	7.34	7.55	13.54	13.12	12.56
Energy gain (kJ $kg^{-0.83}$ day^{-1})	-48.37	-37.64	-33.85	18.38	18.28	27.65	47.56	49.22	83.78	81.93	78.18
Protein gain (g $fish^{-1}$ day^{-1})	-0.101	-0.084	-0.076	0.035	0.038	0.043	0.104	0.111	0.189	0.170	0.154
Protein gain (g $kg^{-0.70}$ day^{-1})	-0.56	-0.47	-0.42	0.179	0.190	0.216	0.50	0.54	0.88	0.80	0.72
Lipid gain (g $fish^{-1}$ day^{-1})	-0.090	-0.060	-0.061	0.056	0.093	0.106	0.167	0.155	0.249	0.241	0.238
FCE				0.33	0.42	0.48	0.46	0.50	0.49	0.49	0.47
Protein gain / DCP intake				0.14	0.15	0.17	0.20	0.22	0.26	0.24	0.21
Energy gain/ DE intake				0.24	0.24	0.36	0.32	0.33	0.41	0.40	0.39
metabolic weight $kg^{0.83}$	0.130	0.131	0.133	0.142	0.148	0.146	0.154	0.153	0.162	0.160	0.161
metabolic weight $kg^{0.70}$	0.179	0.180	0.182	0.193	0.199	0.198	0.207	0.206	0.215	0.213	0.214

Table A 15: *Determination of DE and DCP contents of the 15 diets for trials C, D and E predicted from DE and DCP contents of each ingredient.*

	C1	C2	C3	C4	C5	C6	D7	D8	D9	D10	E11	E12	E13	E14	E15
Composition (g kg^{-1})															
Fish meal A							600	735	885	600					
Fish meal B											870	805	750	700	650
Fish meal C	620	620	620	780	780	780									
Fish oil		40	85		25	65	95	95	95	163	100	150	200	250	300
Cornstarch							285	150		150					
Cellulose	360	320	275	200	175	135				67					
predicted CP g kg^{-1}	434	434	434	546	546	546	458	561	675	458	553	512	477	445	413
predicted DCP g kg^{-1}	360	360	360	453	453	453	403	493	594	403	487	451	420	392	364
predicted GE MJ kg^{-1}	20.3	21.1	22.1	21.1	21.6	22.5	21.7	22.2	22.9	23.1	22.5	23.1	23.8	24.7	25.5
predicted DE MJ kg^{-1}	11.2	12.7	14.4	14.1	15.0	16.5	19.1	19.8	20.6	19.7	20.1	20.8	21.6	22.5	23.4
ADC (%) of protein	83.0	83.0	83.0	83.0	83.0	83.0	87.9	87.9	87.9	87.9	88.1	88.1	88.1	88.1	88.1
ADC (%) of energy	55.4	60.2	65.1	66.9	69.6	73.5	88.1	89.1	90.1	85.4	89.4	90.0	90.6	91.1	91.6
Analysis (g kg^{-1}) DM															
GE (MJ)	19.50	20.60	21.36	20.55	20.70	21.97	21.50	22.10	23.38	23.00	22.64	23.25	23.97	23.98	24.88
CP	446	445	436	543	543	538	461	559	676	463	560	512	470	444	406
DCP	370	369	362	451	451	447	406	492	595	407	493	451	414	391	357
DE	10.80	12.40	13.91	13.70	14.40	16.15	18.95	19.69	21.07	19.64	20.23	20.92	21.71	21.85	22.79
DCP/DE ratio	34.3	29.8	26.0	32.9	31.3	27.6	21.9	25.2	28.2	20.7	24.3	21.5	19.1	17.9	15.7

Table A 16: *Composition (kg^{-1} WW) of fish of Trials C , D and E.*

	Weight g fish^{-1}	Dry matter g	Protein g	Lipid g	Ash g	Energy MJ
Initial	17.45	325	189	87	51	7.59
C1	41.89	289	174	74	44	6.64
C1	42.59	284	172	65	50	6.15
C1	41.29	293	177	66	50	6.41
C2	44.94	304	191	78	45	6.98
C2	51.94	312	185	74	52	6.82
C2	52.94	300	176	90	40	7.47
C3	64.33	307	171	86	40	7.33
C3	54.17	335	189	80	55	7.43
C3	57.13	338	183	102	46	8.39
C4	66.50	333	191	90	55	8.29
C4	76.06	308	185	75	50	6.84
C4	69.11	313	187	68	61	6.52
C5	75.22	330	189	97	43	8.29
C5	68.72	331	187	88	48	7.57
C5	69.89	324	177	99	42	7.90
C6	72.94	339	190	105	42	8.45
C6	70.61	339	188	98	53	8.28
C6	68.41	338	184	104	43	7.91
Initial	25.34	294	177	68	47	6.52
D7	77.68	349	172	140	37	9.12
D7	76.68	335	172	128	37	8.59
D7	77.20	336	172	128	39	8.82
D8	88.50	339	174	137	35	8.76
D8	88.15	359	179	148	37	9.54
D8	90.85	331	178	124	38	8.57
D9	93.40	353	180	145	38	9.09
D9	96.20	348	178	139	39	8.80
D9	96.16	340	180	126	40	8.59
D10	93.50	359	172	145	38	9.18
D10	92.80	354	171	157	38	9.20
D10	94.21	356	178	147	40	9.10

Table A16 continued

	Weight g fish^{-1}	Dry matter g	Protein g	Lipid g	Ash g	Energy MJ
Initial	31.94	294	173	75	45	6.55
E11	105.17	333	173	124	42	8.60
E11	99.06	348	180	124	40	8.63
E11	108.61	366	188	139	47	9.01
E12	107.47	338	172	125	40	8.40
E12	105.21	351	179	127	44	8.72
E12	115.76	357	175	149	42	8.93
E13	103.71	350	169	142	41	8.94
E13	103.00	337	165	126	41	8.77
E13	100.53	366	174	156	39	9.07
E14	95.53	344	165	140	39	8.78
E14	106.72	364	165	153	37	9.54
E14	96.06	361	163	153	45	9.15
E15	89.65	369	165	166	41	9.63
E15	92.74	359	159	163	41	9.16
E15	101.39	368	165	166	38	9.40

***Table A 17**: Feed intake and retention of energy and protein of fish in Trial C after 140 days of growth.*

	C1	C1	C1	C2	C2	C2	C3	C3	C3	C4	C4	C4	C5	C5	C5	C6	C6	C6
Weight initial (g)	17.06	16.94	17.11	17.06	17.5	17.78	18.5	16.61	17.83	17.61	18.11	18.17	17.5	17.17	17.5	16.78	16.89	18.06
Weight final (g)	41.89	42.59	41.29	44.94	51.94	52.94	64.33	54.17	57.13	66.5	76.06	69.11	75.22	68.72	69.89	72.94	70.61	68.41
Average weight (g)	26.73	26.86	26.58	27.69	30.15	30.68	34.50	30.00	31.92	34.22	37.11	35.44	36.28	34.35	34.97	34.98	34.53	35.15
Food fed (g $fish^{-1}$ day^{-1})	0.379	0.388	0.376	0.422	0.446	0.517	0.587	0.523	0.592	0.521	0.574	0.559	0.593	0.557	0.585	0.575	0.571	0.596
Weight gain (g $fish^{-1}$ day^{-1})	0.177	0.183	0.173	0.199	0.246	0.251	0.327	0.268	0.281	0.349	0.414	0.364	0.412	0.368	0.374	0.401	0.384	0.360
FCE	0.47	0.47	0.46	0.47	0.55	0.49	0.56	0.51	0.47	0.67	0.72	0.65	0.70	0.66	0.64	0.70	0.67	0.60
DE fed (kJ $fish^{-1}$ day^{-1})	4.09	4.19	4.06	5.23	5.53	6.41	8.15	7.27	8.23	7.13	7.86	7.66	8.53	8.03	8.43	9.32	9.25	9.65
Energy gain (kJ $fish^{-1}$ day^{-1})	1.06	0.95	0.96	1.32	1.58	1.86	2.36	1.97	2.46	2.98	2.73	2.23	3.51	2.78	2.99	3.49	3.26	2.89
DP fed (g $fish^{-1}$ day^{-1})	0.140	0.144	0.139	0.156	0.165	0.191	0.212	0.189	0.214	0.235	0.259	0.252	0.267	0.251	0.263	0.256	0.254	0.265
Protein gain (g $fish^{-1}$ day^{-1})	0.029	0.029	0.029	0.038	0.045	0.043	0.054	0.051	0.051	0.067	0.076	0.068	0.078	0.069	0.065	0.076	0.072	0.066
Lipid gain (g $fish^{-1}day^{-1}$)	0.012	0.009	0.009	0.014	0.017	0.023	0.028	0.021	0.031	0.032	0.029	0.022	0.041	0.033	0.039	0.044	0.039	0.040
Energy gain (MJ kg $gain^{-1}$)	5.99	5.20	5.58	6.61	6.43	7.41	7.23	7.36	8.75	8.54	6.61	6.14	8.50	7.56	8.00	8.71	8.50	8.02
Protein gain (g kg $gain^{-1}$)	163.7	160.8	168.5	192.2	183.0	169.4	163.7	189.0	180.3	191.7	183.7	186.3	189.0	186.3	173.0	190.3	187.7	182.2
Lipid gain (g kg $gain^{-1}$)	65.1	50.5	51.1	72.5	67.4	91.5	85.6	76.9	108.8	91.1	71.2	61.2	100.0	88.3	103.0	110.4	101.5	110.1
MBW $kg^{-0.83}$	0.049	0.050	0.049	0.051	0.055	0.055	0.061	0.054	0.057	0.061	0.065	0.063	0.064	0.061	0.062	0.062	0.061	0.062
MBW $kg^{-0.70}$	0.079	0.080	0.079	0.081	0.086	0.087	0.095	0.086	0.090	0.094	0.100	0.097	0.098	0.094	0.096	0.096	0.095	0.096
Feed intake (g $kg^{-0.70}$ day^{-1})	4.78	4.88	4.76	5.19	5.17	5.92	6.19	6.09	6.60	5.53	5.76	5.79	6.04	5.90	6.12	6.02	6.02	6.21
Weight gain (g $kg^{-0.70}$ day^{-1})	2.24	2.30	2.19	2.45	2.85	2.88	3.46	3.12	3.13	3.71	4.15	3.77	4.20	3.90	3.91	4.19	4.05	3.75
DE fed (kJ $kg^{-0.83}$ day^{-1})	82.66	84.35	82.45	102.59	101.10	115.53	133.36	133.50	143.62	117.46	121.04	122.55	133.85	131.72	136.26	150.69	151.10	155.38
Energy gain (kJ $kg^{-0.83}$ day^{-1})	21.46	19.17	19.55	25.82	28.92	33.54	38.68	36.26	42.86	49.11	42.08	35.72	54.98	45.71	48.43	56.46	53.28	46.47
DCP fed (g $kg^{-0.70}$ day^{-1})	1.77	1.81	1.76	1.92	1.91	2.19	2.24	2.20	2.39	2.49	2.60	2.61	2.72	2.66	2.75	2.68	2.68	2.76
Protein gain (g $kg^{-0.70}$ day^{-1})	0.37	0.37	0.37	0.47	0.52	0.49	0.57	0.59	0.56	0.71	0.76	0.70	0.79	0.73	0.68	0.80	0.76	0.68

Table A 18: *Feed intake and retention of energy and protein of fish in Trial D after 94 days of growth.*

	D7	D7	D7	D8	D8	D8	D9	D9	D9	D10	D10	D10
Weight initial (g)	25.22	25.33	24.88	25.49	25.36	25.57	24.71	25.42	25.85	25.61	25.00	25.60
Weight final (g)	77.68	76.68	77.20	88.50	88.15	90.85	93.40	96.20	96.16	93.50	92.80	94.21
Average weight (g)	44.26	44.07	43.83	47.50	47.28	48.20	48.04	49.45	49.86	48.93	48.17	49.11
Food fed (g $fish^{-1}$ day^{-1})	0.818	0.840	0.815	0.845	0.857	0.858	0.821	0.838	0.878	0.864	0.843	0.856
Weight gain (g $fish^{-1}$ day^{-1})	0.558	0.546	0.557	0.670	0.668	0.694	0.731	0.753	0.748	0.722	0.721	0.730
FCE	0.68	0.65	0.68	0.79	0.78	0.81	0.89	0.90	0.85	0.84	0.86	0.85
DE fed (kJ $fish^{-1}$ day^{-1})	15.53	15.96	15.48	16.65	16.89	16.91	17.33	17.68	18.52	16.93	16.53	16.78
Energy gain (kJ $fish^{-1}$ day^{-1})	5.787	5.250	5.518	6.479	7.187	6.509	7.318	7.243	6.994	7.355	7.349	7.345
DP fed (g $fish^{-1}$ day^{-1})	0.331	0.340	0.330	0.416	0.422	0.422	0.489	0.499	0.522	0.351	0.343	0.348
Protein gain (g $fish^{-1}$ day^{-1})	0.095	0.093	0.094	0.116	0.120	0.124	0.132	0.134	0.135	0.123	0.122	0.130
Lipid gain (g $fish^{-1}day^{-1}$)	0.097	0.086	0.087	0.111	0.120	0.101	0.126	0.124	0.111	0.126	0.137	0.129
Energy gain (MJ kg $gain^{-1}$)	10.37	9.61	9.91	9.67	10.76	9.37	10.01	9.62	9.35	10.18	10.19	10.06
Protein gain (g kg $gain^{-1}$)	169.6	169.5	169.6	172.8	179.8	178.4	181.1	178.4	181.1	170.1	168.8	178.4
Lipid gain (g kg $gain^{-1}$)	174.0	158.1	156.3	165.1	180.0	146.1	172.4	165.2	147.8	174.7	190.6	176.2
MBW $kg^{-0.83}$	0.075	0.075	0.075	0.080	0.079	0.081	0.080	0.082	0.083	0.082	0.081	0.082
MBW $kg^{-0.70}$	0.113	0.112	0.112	0.118	0.118	0.120	0.119	0.122	0.123	0.121	0.120	0.121
Feed intake (g $kg^{-0.70}$ day^{-1})	7.25	7.47	7.27	7.13	7.26	7.17	6.88	6.88	7.16	7.14	7.05	7.06
Weight gain (g $kg^{-0.70}$ day^{-1})	4.95	4.86	4.97	5.66	5.66	5.80	6.12	6.18	6.10	5.97	6.03	6.02
DE fed (kJ $kg^{-0.83}$ day^{-1})	206.57	212.94	207.56	208.81	212.60	209.47	215.31	214.45	223.07	207.10	204.88	204.71
Energy gain (kJ $kg^{-0.83}$ day^{-1})	76.96	70.07	73.98	81.27	90.48	80.65	90.92	87.85	84.26	89.99	91.10	89.60
DCP fed (g $kg^{-0.70}$ day^{-1})	2.94	3.02	2.95	3.51	3.57	3.53	4.09	4.09	4.26	2.91	2.87	2.87
Protein gain (g $kg^{-0.70}$ day^{-1})	0.84	0.82	0.84	0.98	1.02	1.03	1.11	1.10	1.11	1.02	1.02	1.07

Table A 19*: Feed intake and retention of energy and protein of fish in Trial E after 92 days of growth.*

	E11	E11	E11	E12	E12	E12	E13	E13	E13	E14	E14	E14	E15	E15	E15
Weight initial (g)	31.84	31.42	31.58	32.37	32.37	31.68	32.42	32.68	32.89	31.84	31.58	31.32	31.88	31.89	31.37
Weight final (g)	105.17	99.06	108.61	107.47	105.21	115.76	103.71	103.00	100.53	95.53	106.72	96.06	89.65	92.74	101.39
Average weight (g)	57.87	55.79	58.57	58.98	58.36	60.56	57.99	58.02	57.50	55.15	58.05	54.85	53.46	54.38	56.40
Food fed (g fish^{-1} day^{-1})	0.926	0.840	1.023	0.913	0.890	1.092	0.920	0.949	0.962	0.784	0.925	0.856	0.772	0.795	0.895
Weight gain (g fish^{-1} day^{-1})	0.797	0.735	0.837	0.816	0.792	0.914	0.775	0.764	0.735	0.692	0.817	0.704	0.628	0.661	0.761
FCE	0.86	0.87	0.82	0.89	0.89	0.84	0.84	0.81	0.76	0.88	0.88	0.82	0.81	0.83	0.85
DE fed (kJ fish^{-1} day^{-1})	18.71	16.97	20.67	19.09	18.60	22.82	19.96	20.59	20.88	17.09	20.17	18.66	17.60	18.12	20.41
Energy gain (kJ fish^{-1} day^{-1})	7.564	7.055	8.388	7.508	7.667	8.981	7.770	7.492	7.569	6.850	8.818	7.324	7.114	6.963	8.126
DP fed (g fish^{-1} day^{-1})	0.46	0.41	0.50	0.41	0.40	0.49	0.38	0.39	0.40	0.31	0.36	0.33	0.28	0.28	0.32
Protein gain (g fish^{-1} day^{-1})	0.138	0.135	0.163	0.140	0.144	0.161	0.130	0.123	0.128	0.111	0.132	0.111	0.101	0.100	0.123
Lipid gain (g fish^{-1}day^{-1})	0.12	0.11	0.14	0.12	0.12	0.16	0.13	0.11	0.14	0.12	0.15	0.13	0.14	0.14	0.16
Energy gain (MJ kg gain^{-1})	9.49	9.60	10.02	9.20	9.68	9.83	10.03	9.80	10.30	9.89	10.80	10.41	11.33	10.53	10.68
Protein gain (g kg gain^{-1})	173.0	183.3	194.1	171.6	181.7	175.8	167.2	161.3	174.5	161.0	161.6	158.2	160.6	151.7	161.4
Lipid gain (g kg gain^{-1})	145.9	146.1	165.6	147.0	150.7	176.3	172.5	150.0	195.3	171.8	185.4	190.6	216.4	208.4	206.5
MBW kg$^{-0.83}$	0.094	0.091	0.095	0.095	0.095	0.098	0.094	0.094	0.093	0.090	0.094	0.090	0.088	0.089	0.092
MBW kg$^{-0.70}$	0.136	0.133	0.137	0.138	0.137	0.140	0.136	0.136	0.135	0.132	0.136	0.131	0.129	0.130	0.134
Feed intake (g kg$^{-0.70}$ day^{-1})	6.81	6.34	7.46	6.62	6.50	7.77	6.75	6.96	7.10	5.96	6.78	6.53	6.00	6.10	6.70
Weight gain (g kg$^{-0.70}$ day^{-1})	5.86	5.54	6.10	5.92	5.79	6.51	5.69	5.61	5.43	5.26	5.99	5.37	4.88	5.08	5.70
DE fed (kJ kg$^{-0.83}$ day^{-1})	199.17	186.27	217.88	200.04	196.63	233.92	212.15	218.77	223.45	189.39	214.12	207.69	200.11	203.11	221.97
Energy gain (kJ kg$^{-0.83}$ day^{-1})	80.53	77.42	88.42	78.67	81.06	92.07	82.58	79.59	81.01	75.89	93.63	81.51	80.88	78.05	88.38
DCP fed (g kg$^{-0.70}$ day^{-1})	3.36	3.12	3.68	2.99	2.93	3.51	2.80	2.88	2.94	2.33	2.65	2.55	2.14	2.18	2.39
Protein gain (g kg$^{-0.70}$ day^{-1})	1.01	1.02	1.18	1.02	1.05	1.14	0.95	0.90	0.95	0.85	0.97	0.85	0.78	0.77	0.92

Table A 20*: Calculation of partial protein efficiencies (k_{DCPg}) for growth above DCP_m of 0.77g kg$^{-0.70}$ day^{-1} for fish in Trials C, D and E.*

	DCP intake (g kg-0.70 day^{-1})	DCP intake - DCP_m (g kg$^{-0.70}$ day^{-1})	Protein gain (g kg$^{-0.70}$ day^{-1})	k_{DCPg}
C1	1.77	1.00	0.37	0.37
C1	1.81	1.04	0.37	0.36
C1	1.76	0.99	0.37	0.37
C2	1.92	1.15	0.47	0.41
C2	1.91	1.14	0.52	0.46
C2	2.19	1.42	0.49	0.34
C3	2.24	1.47	0.57	0.38
C3	2.20	1.43	0.59	0.41
C3	2.39	1.62	0.56	0.35
C4	2.49	1.72	0.71	0.41
C4	2.60	1.83	0.76	0.42
C4	2.61	1.84	0.70	0.38
C5	2.72	1.95	0.79	0.41
C5	2.66	1.89	0.73	0.39
C5	2.75	1.98	0.68	0.34
C6	2.68	1.91	0.80	0.42
C6	2.68	1.91	0.76	0.40
C6	2.76	1.99	0.68	0.34
D7	2.94	2.17	0.84	0.39
D7	3.02	2.25	0.82	0.37
D7	2.95	2.18	0.84	0.39
D8	3.51	2.74	0.98	0.36
D8	3.57	2.80	1.02	0.36
D8	3.53	2.76	1.03	0.38
D9	4.09	3.32	1.11	0.33
D9	4.09	3.32	1.10	0.33
D9	4.26	3.49	1.11	0.32
D10	2.91	2.14	1.02	0.48
D10	2.87	2.10	1.02	0.48
D10	2.87	2.10	1.07	0.51

Table A20 continued

	DCP intake (g kg-0.70 day^{-1})	DCP intake - DCP_m (g kg$^{-0.70}$ day^{-1})	Protein gain (g kg$^{-0.70}$ day^{-1})	k_{DCPg}
E11	3.36	2.59	1.01	0.39
E11	3.12	2.35	1.02	0.43
E11	3.68	2.91	1.18	0.41
E12	2.99	2.22	1.02	0.46
E12	2.93	2.16	1.05	0.49
E12	3.51	2.74	1.14	0.42
E13	2.80	2.03	0.95	0.47
E13	2.88	2.11	0.90	0.43
E13	2.94	2.17	0.95	0.44
E14	2.33	1.56	0.85	0.54
E14	2.65	1.88	0.97	0.51
E14	2.55	1.78	0.85	0.48
E15	2.14	1.37	0.78	0.57
E15	2.18	1.41	0.77	0.55
E15	2.39	1.62	0.92	0.57

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