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in

Brufau J. (ed.), Tacon A. (ed.).
Feed manufacturing in the Mediterranean region: Recent advances in research and technology

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 37

1999
pages 195-215

Growth and feed utilization of gilthead sea bream, *Sparus aurata,* fed diets with supplementary enzymes

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SUMMARY - A twelve-week experiment investigated the effects of replacing fish meal (FM) with dehulled hexane extracted soybean meal (SBM) and the addition of enzymes on the growth and feed utilization of 50 g gilthead sea bream, *Sparus aurata*. Three levels of hexane extracted dehulled SBM, 220, 320 and 440 g/kg, and FM, 320, 260 and 230 g/kg respectively were used in these pressed diets, and two diets FM two enzyme cocktails were added to the latter to give a total of 7 treatments. Fish were fed to satiation. Growth and feed utilization decreased as the level of SBM in the diet fed to the fish increased. The addition of 1 g/kg of a low pH active protease together with 1 g/kg of an α-galactosidase (α-gal) and of 1 g/kg of a high pH active protease together with 1 g/kg of the α-gal to the diet containing 320 g/kg SBM brought about improvements in all the nutritional parameters studied, significantly so in most cases. The best performance was given by fish fed the former of these two diets, but both also performed better than fish fed the diet containing higher FM. Feeding fish the 440 g/kg SBM diet containing low pH protease and α-gal appeared to improve some of the nutritional parameters compared to the fish fed the unsupplemented diet, but feeding fish the 440 g/kg SBM diet to which the same quantity of high pH active protease and α-gal had been added appeared to reduce the performance compared to fish fed the unsupplemented diet. The results obtained indicate the potential for improving feed performance by the addition of enzymes to the food; however, more work is required to understand the factors affecting the use of such enzymes.

Key words: Fish meal, soybean meal, supplementary enzymes, *Sparus aurata*.

RESUME - *"Croissance et utilisation alimentaire de la dorade,* Sparus aurata, *recevant un régime supplémenté en enzymes". Un essai de douze semaines a étudié les effets du remplacement de la farine de poisson (FP) par une farine de soja décortiqué sans hexane (FS) et l'addition d'enzymes pour la croissance et l'utilisation alimentaire de dorades de 50 g,* Sparus aurata. *Trois niveaux de FS décortiquée sans hexane, 220, 320 et 440 g/kg, et de FP, 320, 260 et 230 g/kg ont été utilisés respectivement pour ces trois régimes pressés, et aux deux derniers régimes on a ajouté deux mélanges d'enzymes pour faire un total de 7 traitements. Les poissons ont été alimentés à satiété. La croissance et l'utilisation alimentaire ont diminué en même temps qu'augmentait le niveau de FS dans le régime offert aux poissons. L'addition de 1 g/kg d'une protéase active à faible pH en même temps que 1 g/kg d'une α-galactosidase (α-gal) et de 1 g/kg d'une protéase active à pH élevé en même temps que 1 g/kg d'une α-gal au régime contenant 320 g/kg de FS a amené des améliorations de tous les paramètres nutritionnels étudiés, de façon significative dans la plupart des cas. Les meilleures performances ont été obtenues par les poissons recevant le premier de ces deux régimes, mais tous deux ont eu de meilleurs résultats que les poissons recevant le régime contenant le plus de FP. Le fait d'alimenter les poissons avec le régime à 440 g/kg de FS qui contenait la protéase active à faible pH et α-gal, s'est avéré améliorer certains des paramètres nutritionnels en comparaison avec les poissons recevant le régime non supplémenté, mais la nutrition des poissons avec le régime à 440 g/kg de FS supplémenté avec la même quantité de protéase active à pH élevé et α-gal, semblait réduire les performances en comparaison avec les poissons recevant le régime non supplémenté. Les résultats obtenus montrent le potentiel qui existe d'amélioration des performances alimentaires par l'addition d'enzymes à l'aliment ; cependant, des travaux ultérieurs sont nécessaires pour comprendre les facteurs qui influencent l'utilisation de ces enzymes.*

Mots-clés : *Farine de poisson, farine de soja, enzymes supplémentaires,* Sparus aurata.

Introduction

The aquaculture industry is currently heavily dependent on the use of fish meal (and fish oil). In 1994 aquaculture used 15.5% of the total production of fish meal (FAO, 1996) and its use is expected

to increase in order to keep up with the increasing level of production and intensification in the aquaculture industry.

Although a lot of effort has been applied to improve the quality of the fish meal available, increasing prices and the restricted availability has enhanced the amount of work being carried out to find alternative protein sources. Among sources of protein investigated, with varying degrees of success, are fish silage (Stone *et al.*, 1989), krill (*Euphausia superba*)(Lou and Chen, 1980), terrestrial worms (Tacon *et al.*, 1983), single cell protein (Davies and Wareham, 1988), pito brewery waste (Oduro-Boateng and Bart-Plange, 1988), green alga (*Cladophora glomerata*)(Appler and Jauncey, 1983), animal products (Alexis, 1997), plant products (Webster *et al.*, 1992a) and combinations of animal and plant products (El-Sayed, 1994).

From the point of view of economics, market availability and nutritional value, a prime candidate for replacing fish meal (FM) in aquaculture diets is soybean meal (SBM; *Glycine max* L.). Although the protein content of SBM is less than that of FM, the essential amino acid profiles of processed SBM products compare well with that of FM when considered on a percentage of protein basis (NRC, 1993) and, with the exception of methionine, would seem to meet the requirements of channel catfish *Ictalurus punctatus* (NRC, 1993), Chinook salmon *Oncorhynchus tshawytscha* (NRC, 1993) and gilthead sea bream *Sparus aurata* (Deguara, 1998). Notwithstanding the favourable nutritional composition of SBM and of other plant materials, their use is often restricted by the presence of the numerous antinutritional factors they contain. These antinutritional factors can be divided into two groups, those that are heat-labile (such as the proteolytic enzyme inhibitors) and those that are heat-stable (such as the oligosaccharides).

The poultry and pig industries have for some time been using supplemental enzymes in their feeds. The use of these enzymes has shown that lower cost raw ingredients or cheaper less-processed materials can be used with equal and even better performance than more expensive materials, thereby increasing the choice and flexibility of the feed manufacturer. These enzymes are generally obtained from bacteria, yeasts or moulds and often used in combinations (cocktails) although one particular enzyme may be the main component. The enzymes have been reported to have a beneficial effect in broiler chickens (Bedford and Classen, 1992; Walsh and Headon, 1994; Finnfeeds 1995a,b,c), layers (Nasi, 1988; Graham and Bedford, 1992), turkeys (Salmon *et al.*, 1986), starter pigs (Thomke *et al.*, 1980; Graham *et al.*, 1988; Finnfeeds, 1996a,b) and larger swine (Classen *et al.*, 1991; Graham and Inborr, 1993).

In this experiment the effect of replacing FM with SBM in pressed gilthead sea bream, *Sparus aurata*, diets was investigated. In addition, two enzyme cocktails were added to each of the higher SBM containing diets in order to investigate whether supplementary enzymes had an influence on fish performance and which of the two cocktails used had the most beneficial effect.

Materials and methods

Experimental tanks

This experiment was carried out in the aquarium facilities of the National Aquaculture Centre (NAC) in Malta. 21 Fibreglass tanks were utilized in this experiment. The volume of water in these tanks was 0.27 m^3 (length, 0.9 m, width, 0.6 m, depth, 0.5 m). Borehole water was supplied by an inlet pipe, connected to a ring circuit to ensure equal pressure throughout the system, at a rate of 6 L/min, such that complete water exchange was achieved in about 45 minutes.

Before the start of the experiment the tanks were randomly allocated to the different treatments such that each treatment had three replicates.

Experimental fish and handling

50 g gilthead sea bream were obtained from a commercial fish farm situated in the Maltese Islands. After arrival the fish were randomly distributed into the tanks for the acclimation period.

45 were put into each of the tanks for the acclimation period but only 40 used during the experiment itself. The duration of the experiment was 12 weeks.

At the beginning of each experiment, prior to weighing, 10 fish were sacrificed for determination of condition factor and hepatosomatic index and another 10 for pooled carcass analysis. These fish were randomly taken from all the experimental tanks. The fish in each tank were weighed and extra fish removed. All fish were weighed in bulk every two weeks until the end of the experiment, and any mortalities were recorded.

After the final weighing, 5 fish from tanks fed the same diet were randomly taken for the determination of condition factor and hepatosomatic index and another 5 taken for pooled carcass analysis.

The diets and feeding regime

The diets used in the experiments were formulated, using a RAPP least cost feed formulation program (ATH Matematik-Konsult, Korsfararvagen 140, S-181 40 Lidingo, Sweden), and manufactured by Ewos SA (N-620, km 99, Duenas, Palencia 34210) in Spain with a Norvidan double-pelletiser type pellet mill.

The enzymes used were supplied by Finnfeeds International (P.O. Box 777, Marlborough, Wiltshire, SN8 1XN) in England. Three enzymes were used in the experiments:

A protease with a pH optimum at 3.0. This is designated as 'low pH protease' in the text.
A protease with a pH optimum at 8.5. This is designated as 'high pH protease' in the text.
An α-galactosidase with a pH optimum at 5.0. This is designated as 'α-gal' in the text.

These enzymes had temperature optima between 50 and 60°C.

Dry enzymes (with wheat as the carrier) were added to the feed mix prior to pelleting, and liquid enzymes (in aqueous solution) were mixed with oil and sprayed onto the pellets post-extrusion.

The formulations of the diets used in this experiment are given in Table 1. The nutritional composition and essential amino acid contents (except tryptophan) of the SBM and FM used are given in Table 2. The percentage inclusion levels of the supplementary enzymes added to the diets and the nutritional composition of the diets themselves (3 mm pellets) are given in Table 3. Table 4 gives the essential amino acid contents (except tryptophan) of 63 g gilthead sea bream carcass and the diets used in this experiment.

During the acclimation period of two weeks the fish were fed a mixture of all the feeds. The fish were fed twice daily at 08:30 and 16:00. Fish were fed 6 days of the week to satiation (determined as the point at which the fish did not consume offered pellets after 5 minutes).

Water quality and environmental parameters

The dissolved oxygen level was maintained above 5.0 mg/L throughout the experiment. Temperatures varied from 21.5 to 22.4°C throughout the experiment. The levels of ammonia, nitrite and nitrate did not go above 0.6, 0.15 and 10 mg/L respectively at any point during the experiment. The photoperiod maintained throughout the experiment was L:D 12:12, operated by a fully automated switching system. Light came on at 06:30 and switched off at 18:30.

Faecal collection

Faeces for digestibility studies were collected during weeks 7 and 8 and were pooled. Faeces were collected hourly using a 1.5 mm plastic mesh inserted into the central pipe. These faeces were dried at 105°C and kept in a desiccator until analysed.

Apparent Digestibility Coefficients (ADC) were calculated using the following formula:

ADC (%) = 100 - {100 * (% Cr_2O_3 in food/% Cr_2O_3 in faeces) * (% nutrient in faeces/% nutrient in food)}; all calculations were based on dry matter values.

Table 1. Formulations of diets used (Inclusion g/kg)

	Diets			Source
	220C	320C, 320L, 320H	440C, 440L, 440H	
Fish meal	317	260	229	Spain
Dehulled hexane extracted soybean meal	220	320	440	Spain
Blood meal	50	33	0	Daka Ltd., Canada
Corn	88	58	0	Suprex Ltd., Scotland
Feather meal	100	100	76	Canada
Fish oil	64	79	100	UFP Ltd., Scotland
Limestone	30	30	30	
Molasses	40	60	60	Spain
Vitamins and minerals	11	11	11	Ewos Premix prepared by Roche Products Ltd., England
Whole wheat	75	45	45	Scotland
Chromic oxide	5	5	5	

Table 2. Nutritional compositions of the soybean meal and fish meal used

	Soybean meal	Fish meal
Moisture (g/kg)	126	75
Crude protein (g/kg)	486	654
Crude lipid (g/kg)	7	73
Ash (g/kg)	58	168
Crude fibre (g/kg)	34	5
Crude carbohydrate (g/kg)	300	16
Phosphorus (g/kg)	7	23
Protein solubility (%)	79.37	
Trypsin inhibitor activity (mg/g)	1.11	
Essential amino acid (g/100 g protein)		
Arginine	4.83	6.23
Histidine	1.85	3.50
Isoleucine	3.17	4.98
Leucine	4.98	7.73
Lysine	4.22	8.73
Methionine	0.70	2.31
Cystine	1.06	1.54
Phenylalanine	3.30	4.26
Tyrosine	1.67	3.40
Threonine	2.91	4.52
Valine	3.15	5.58

Table 3. Inclusion levels of enzymes and nutritional compositions of the diets

	Diet						
	220C	320C	320L	320H	440C	440L	440H
Fish meal inclusion (g/kg)	320	260	260	260	230	230	230
Soybean meal inclusion (g/kg)	220	320	320	320	440	440	440
Low pH protease (g/kg)	0	0	1	0	0	1	0
High pH protease (g/kg)	0	0	0	1	0	0	1
α-gal (g/kg)	0	0	1	1	0	1	1
Enzyme form	Dry	Dry	Dry	Dry	Dry	Dry	Liquid
Moisture (g/kg)	81	84	84	84	87	81	86
Crude protein (g/kg)	485	476	470	464	459	456	459
Crude lipid (g/kg)	129	128	124	124	128	142	110
Ash (g/kg)	114	116	100	113	112	103	109
Crude fibre (g/kg)	19	15	17	20	24	19	21
Crude carbohydrate (g/kg)	178	183	197	193	182	200	214
Phosphorus (g/kg)	12	11	10	10	11	10	10
Trypsin inhibitor activity (mg/g)	0.28	0.24	0.34	0.14	0.29	0.17	0.54
Chromic oxide (g/kg)	5	5	5	5	5	5	5
Energy content (kJ/g)	22.03	21.70	21.77	21.39	21.37	21.43	20.98
Protein/gross energy ratio (g/MJ)	22.00	21.93	21.60	21.67	21.49	21.27	21.89

Table 4. Essential amino acid contents of gilthead sea bream carcass† and the diets

	Carcass	Diet						
		220C	320C	320L	320H	440C	440L	440H
Fish meal inclusion (g/kg)		320	260	260	260	230	230	230
Soybean meal inclusion (g/kg)		220	320	320	320	440	440	440
Low pH protease (g/kg)		0	0	1	0	0	1	0
High pH protease (g/kg)		0	0	0	1	0	0	1
α-gal (g/kg)		0	0	1	1	0	1	1
Essential amino acid (g/100g protein)								
Arginine	5.10	4.28	4.70	5.23	6.57	4.89	4.05	4.52
Histidine	1.83	2.03	2.31	2.26	2.25	1.97	1.94	2.17
Isoleucine	3.62	2.78	2.79	3.10	2.90	2.86	2.27	3.08
Leucine	5.83	6.65	6.57	6.88	6.91	6.42	5.60	5.84
Lysine	6.01	5.04	4.84	4.86	5.14	5.18	4.98	4.61
Methionine	2.56	0.56	0.49	0.60	0.64	0.71	0.72	0.53
Cystine††	1.08	0.95	1.11	1.19	1.20	1.09	1.10	1.06
Phenylalanine	3.26	4.13	4.19	4.25	4.18	3.99	3.95	4.05
Tyrosine††	3.17	1.99	2.08	2.50	2.41	2.24	2.17	2.22
Threonine	3.91	3.35	3.60	3.78	3.83	4.69	3.57	3.70
Valine	4.39	3.23	3.51	3.61	3.57	3.11	3.15	3.46

†Average weight 63.28 g
††Non essential amino acid

Intestinal dry matter contents

Intestinal dry matter contents of 6 fish from each diet were determined. The fish sampled for this purpose were killed in lethal anaesthetic (0.6 mL/L 2-phenoxyethanol), dissected while still fresh and the intestinal contents collected from the point immediately behind the pyloric caeca to the anus by forcing out the contents with a pair of pincers. The collected samples were then dried at 105°C.

Laboratory analysis

Chemical analysis

Fish sampled for pooled chemical analysis were frozen and then thawed before blending (whole). Representative samples of each feed were randomly taken from the feed sacks and then ground and material which passed through a 1 mm sieve used for analysis. Crude protein, crude lipid, moisture, ash, crude fibre and phosphorus analyses were performed according to standard methods of the AOAC (1990) and ISO (1978, 1981, 1983). 3,5-Dinitrosalicylic acid was used to determine crude carbohydrate content (James, 1995). The chromic oxide content of feeds and faeces was analysed by Atomic Absorption Spectroscopy (on a Varian AA-1275 Series). Trypsin inhibitor activity was determined by the method of Smith *et al.* (1980) and protein solubility of the soybean meal by the method of Araba and Dale (1990). Amino acid analysis was carried out on an LKB Biochrom 4151 Alpha plus amino acid analyser (column used was an Ultiopac8 cation-exchange resin, 202 x 4.6 mm internal diameter), and energy content on a Gallenkamp Autobomb.

Fish condition factor and hepatosomatic index

The fish sampled for these parameters were analysed while still fresh.

The Condition Factor of the fish was determined using the following formula:
Condition Factor = 100 * fish weight (g)/(total length in cm^3).

The Hepatosomatic Index (HI) was calculated using the following formula:
Hepatosomatic index = 100 * liver weight (g)/body weight (g).

Assessment of growth and feed utilization

Growth and feed performance were described using the parameters, all calculations based on an as fed, wet basis.

Specific Growth Rate (SGR)(%/day) = 100 * (Log_efinal weight (g) - Log_einitial weight (g)/number of days.
Food Conversion Ratio (FCR) = food given (g)/increase in biomass of fish (g).
Protein Efficiency Ratio (PER) = increase in biomass of fish (g)/protein intake (g).
Apparent Net Protein Utilization (ANPU)(%) = 100 * protein deposition (g)/protein intake (g).
Apparent Net Lipid Utilization (ANLU)(%) = 100 * lipid deposition (g)/lipid intake (g).
Energy Efficiency (EE)(%) = 100 * energy deposition (kJ)/energy intake (kJ).

Statistical analysis

The results from the replicates for each treatment were used to provide the data for the statistical analysis. Homogeneity of variances between samples was tested using the Levene's test (Dixon *et al.*, 1988). Multiple comparisons between means were made using the Student-Newman-Keuls test. All percentage and ratio data were transformed to arcsine values prior to analysis (Zar, 1984). In the case where a homogeneity of variances was not found, the nonparametric Kruskal-Wallis test was performed. The significance levels of the tests was taken as 0.05. Statistical analysis was performed using the BMDP statistical software package (Version PC90).

Results

Assessment of growth and feed performance

Going from a low content of SBM to a high level of SBM (diet 220C to 320C to 440C) a clear decrease in performance was seen in this experiment in terms of fish growth and feed utilization, although this was not always significant (Table 5). The SGR, ANLU and EE of fish fed diets 220C and 320C were both significantly higher than that of fish fed diet 440C. Fish fed diet 220C also showed significantly higher ANPU and EE than fish fed diet 320C.

Table 5. Assessment of growth and feed performance[†]

	Diet						
	220C	320C	320L	320H	440C	440L	440H
Fish meal inclusion (g/kg)	320	260	260	260	230	230	230
Soybean meal inclusion (g/kg)	220	320	320	320	444	444	444
Low pH protease (g/kg)	0	0	1	0	0	1	0
High pH protease (g/kg)	0	0	0	1	0	0	1
α-gal (g/kg)	0	0	1	1	0	1	1
Initial weight (g)	52.42^{a} (1.84)	51.75^{a} (1.52)	51.79^{a} (1.98)	49.63^{a} (3.20)	51.67^{a} (2.03)	50.80^{a} (0.14)	50.38^{a} (2.06)
Final weight (g)	86.60^{cd} (1.17)	80.53^{bc} (2.12)	98.80^{e} (7.02)	89.61^{d} (5.20)	72.05^{b} (4.44)	73.39^{b} (3.62)	57.68^{a} (3.31)
Specific growth rate (SGR) (%/day)	0.60^{c} (0.06)	0.53^{c} (0.04)	0.77^{d} (0.04)	0.71^{d} (0.03)	0.39^{b} (0.03)	0.44^{b} (0.06)	0.16^{a} (0.05)
Mortalities (%)	1.67^{a} (2.89)	0.00^{a} (0.00)	0.00^{a} (0.00)	0.83^{a} (1.44)	4.17^{a} (1.44)	0.83^{a} (1.44)	0.83^{a} (1.44)
Food intake (g/100g fish/day)	1.78^{b} (0.14)	1.82^{b} (0.05)	1.98^{c} (0.05)	2.07^{c} (0.07)	1.56^{a} (0.07)	1.79^{b} (0.09)	1.52^{a} (0.10)
Food conversion ratio (FCR)	2.50^{a} (0.22)	2.82^{a} (0.11)	2.18^{a} (0.07)	2.46^{a} (0.06)	3.30^{a} (0.23)	3.50^{a} (0.59)	8.09^{b} (2.59)
Protein efficiency ratio (PER)	0.83^{c} (0.08)	0.75^{bc} (0.03)	0.98^{d} (0.03)	0.88^{cd} (0.02)	0.66^{b} (0.05)	0.64^{b} (0.10)	0.29^{a} (0.08)
Apparent net protein utilization (ANPU)(%)	15.64^{cd} (1.37)	14.23^{bc} (0.46)	18.28^{e} (0.48)	16.68^{de} (1.12)	12.56^{b} (0.74)	13.81^{bc} (1.78)	6.38^{a} (1.29)
Apparent net lipid utilization (ANLU)(%)	24.10^{d} (3.52)	16.60^{c} (1.73)	46.29^{f} (1.73)	34.30^{e} (5.81)	0.67^{b} (3.10)	3.01^{b} (3.67)	-23.96^{a} (5.44)
Energy efficiency (EE) (%)	15.47^{d} (1.69)	12.57^{c} (0.71)	22.08^{f} (0.66)	17.85^{e} (0.46)	7.27^{b} (1.21)	8.36^{b} (2.02)	-1.91^{a} (1.80)

[†]Data are presented as means with the standard deviation in brackets
a,b,c,d,e,f: Means in a row followed by the same superscript are not significantly different ($P<0.05$)

The inclusion of low pH protease and α-gal at the 320 g/kg SBM level (diet 320L) significantly improved fish performance over the fish fed diet 320C, which contained the same amount of SBM but no enzymes, and over fish fed diet 220C in SGR, PER, ANPU, ANLU and EE. Fish fed diet 320H, containing high pH protease and α-gal, also showed significantly better results for SGR, ANPU, ANLU and EE than fish fed diets 220C and 320C. Feeding of both diet 320L and 320H gave fish performances which were significantly better than when the fish were fed diets 440C, 440L and 440H

in all the above parameters. Feeding of diet 320L also resulted in significantly better ANLU and EE than feeding fish diet 320H.

Fish fed diets 320L and 320H ate significantly more food than fish fed any of the other diets, and fish fed diets 220C, 320C and 440L ate significantly more than fish fed either diet 440C or 440H. The only significant differences in FCR was the higher value given by fish fed diet 440H. However, the results given by fish fed the other diets followed the same trends seen above, the best result being given by fish fed diet 320L followed by fish fed diet 320H.

The significant impact of enzyme addition to the 320 g/kg SBM diet was not mirrored to the same extent in the 440 g/kg SBM diet. Fish fed diet 440L did show a slight improvement in SGR, ANPU, ANLU and EE, but not in FCR or PER, and fish fed diet 440H gave the worst results for all parameters with only an average growth of 7.3 g over the whole experimental period and a negative ANLU and EE.

Carcass composition, condition factor, hepatosomatic index and intestinal dry matter content

There was an increase in carcass ash, moisture, phosphorus and protein content, along with a decrease in carcass lipid and energy content of the fish, by the end of the experiment (Table 6).

Table 6. Effect of dietary treatments on the body composition of fish

	Initial	Diet						
		220C	320C	320L	320H	440C	440L	440H
Fish meal inclusion (g/kg)		320	260	260	260	260	260	260
Soybean meal inclusion (g/kg)		220	320	320	320	320	320	320
Low pH protease (g/kg)		0	0	1	0	0	1	0
High pH protease (g/kg)		0	0	0	1	0	0	1
α-gal (g/kg)		0	0	1	1	0	1	1
Moisture (g/100g)†	64.60	65.74	66.75	64.79	66.55	67.35	67.24	67.40
Protein (g/100g)†	16.28	17.27	17.29	17.41	17.73	17.02	17.89	17.06
Lipid (g/100g)†	14.55	11.89	11.49	13.58	12.36	10.67	10.63	10.19
Ash (g/100g)†	3.71	4.22	4.25	3.90	4.07	4.35	4.17	4.07
Phosphorus (g/100g)†	0.66	0.76	0.74	0.68	0.71	0.73	0.72	0.69
Energy content (kJ/g)†	9.97	9.32	9.04	9.65	9.22	8.88	9.05	8.92
Condition factor††	1.48^{a} (0.16)	1.46^{a} (0.09)	1.42^{a} (0.06)	1.49^{a} (0.03)	1.44^{a} (0.13)	1.38^{a} (0.04)	1.29^{a} (0.13)	1.32^{a} (0.15)
Hepatosomatic index††	1.73^{b} (0.41)	1.32^{ab} (0.19)	1.41^{ab} (0.24)	1.37^{ab} (0.14)	1.40^{ab} (0.33)	1.34^{ab} (0.32)	1.20^{a} (0.21)	1.09^{a} (0.30)
Intestinal dry matter content (g/100g)††		18.12^{bc} (3.14)	19.69^{c} (3.19)	15.60^{ab} (3.28)	14.04^{ab} (2.83)	15.03^{ab} (1.49)	15.03^{ab} (1.45)	12.51^{a} (3.57)

†Values are averages of pooled carcass samples
††Condition factor, hepatosomatic index and final intestinal percentage dry matter content are presented as means with the standard deviation in brackets
a,b,c: Means in a row followed by the same superscript are not significantly different ($P<0.05$)

As the level of SBM in the formulation increased (diets 220C, 320C and 440C), without addition of enzymes, the fish fed these diets showed an increase in carcass moisture level and a corresponding decrease in carcass lipid level. Feeding of the diets containing 440 g/kg SBM, with and without enzymes (diets 440C, 440L and 440H), resulted in fish having the highest carcass moisture contents and the lowest lipid contents. Fish fed diet 320L containing 320 g/kg SBM and low pH protease and α-gal showed the lowest carcass moisture content of all the experimental fish, but the highest lipid content.

There were also decreases in fish condition factor and hepatosomatic indexes (HSI) by the end of the experiment. A decrease in the condition factor of fish was seen as the SBM level of the diets fed went up from 220 g/kg to 440 g/kg although this was not significant, and there was also a decrease in HSI of the fish fed diets containing 440 g/kg SBM (diets 440C, 440L and 440H) compared to fish fed diets containing 320 g/kg SBM (diets 320C, 320L And 320H), again not being significantly so.

As regards the intestinal dry matter contents of the guts, fish fed diet 320C gave the highest value, being significantly different from fish fed all the other diets except diet 220C, which in turn was significantly higher than that found in fish fed diet 440H. Feeding 320 g/kg SBM diets with enzymes to the fish (diets 320L and 320H) reduced the intestinal dry matter content compared to fish fed the unsupplemented diet 320C. A similar reduction in intestinal dry matter content was also observed in fish fed the high pH protease and α-gal supplemented diet 440H compared to fish fed the unsupplemented diet 440C.

Apparent digestibility coefficients

Fish fed diet 320L, containing 320 g/kg SBM with supplemental low pH protease and α-gal, gave the best ADCs for protein, lipid, organic matter, energy and phosphorus of all the diets, followed by fish fed diet 320H in lipid, organic matter and phosphorus ADCs (Table 7). The carbohydrate ADC was the highest in fish fed diet 440H and the lowest ADCs for all nutritional components were given by fish fed diet 440L. All diets fed except diet 440L gave higher protein ADCs than the fish fed the 220 g/kg SBM diet 220C.

Table 7. Effect of dietary treatments on apparent digestibility coefficients (ADC) calculated using faeces collected during weeks 7 and 8†

	Diet						
	220C	320C	320L	320H	440C	440L	440H
Fish meal inclusion (g/kg)	320	260	260	260	260	260	260
Soybean meal inclusion (g/kg)	220	320	320	320	320	320	320
Low pH protease (g/kg)	0	0	1	0	0	1	0
High pH protease (g/kg)	0	0	0	1	0	0	1
α-gal (g/kg)	0	0	1	1	0	1	1
Protein ADC (%)	84.54	86.97	87.73	86.52	85.58	81.24	85.34
Lipid ADC (%)	94.22	94.59	97.14	96.20	93.69	91.90	95.16
Carbohydrate ADC (%)	86.68	77.06	84.66	82.25	71.12	72.16	92.05
Energy ADC (%)††	88.83	88.67	90.78	89.62	87.13	84.08	90.19
Organic matter ADC (%)	67.34	73.27	78.76	77.96	67.16	62.59	74.78
Phosphorus ADC (%)	43.86	45.66	59.52	53.68	23.80	5.67	28.24

†Values are averages of pooled faecal samples
††Energy calculated using the following values: protein, 23.4 kJ/g; lipid, 39.8 kJ/g; carbohydrate, 17.2 kJ/g

The phosphorus ADCs shown by the fish fed diets containing 440 g/kg SBM were all lower than that shown by fish fed the 220 g/kg SBM diet 220C, while fish fed diets containing 320 g/kg showed the highest values of all the diets used.

The carbohydrate ADC of the fish decreased as the level of dietary SBM increased (diets 220C, 320C and 440C). Feeding fish the 320 g/kg SBM enzyme supplemented diets 320L and 320H improved the ADC of lipid, carbohydrate, organic matter, energy and phosphorus compared to fish fed the unsupplemented diet 320C. On the other hand, feeding fish enzyme supplemented 440 g/kg SBM diets (diets 440L and 440H) gave mixed results when compared to the fish fed the unsupplemented diet 440C. From these three diets, fish fed diet 440H gave higher ADC values for all components except protein, while fish fed diet 440L gave lower ADCs in all parameters except carbohydrate.

Discussion

For the purpose of simplification, the discussion of this experiment has been divided into two parts, the first discussing the impact of increasing the SBM level in the diet at the expense of FM, and the second analysing the effect of adding the enzyme mixes on the performance of the fish.

Replacing fish meal in gilthead sea bream diets

As the level of SBM was increased from 220 g/kg (diet 220C) to 320 g/kg (diet 320C) to 440 g/kg (diet 440C) a progressive reduction in fish performance was observed in all the nutritional parameters studied with a number of significant differences being obtained. While no significant differences were recorded in food conversion ratio (FCR) between fish fed these diets, the values themselves vary a lot, with fish fed diet 320C giving an FCR 113% and fish fed diet 440C 132% of the FCR given by fish fed diet 220C.

The work of other authors in which SBM has been used to replace FM in gilthead sea bream diets did not show the same decreasing trend in fish performance as seen in this experiment. Instead, a number of these trials have shown a positive effect of replacing FM with SBM. Millan *et al.* (1989), working with 75 g *Sparus aurata* obtained better growth and feed utilization with a diet containing 450 g/kg SBM (200 g/kg FM) than with the control diet containing 0 g/kg SBM and 475 g/kg FM. This diet gave a better fish performance than did the other experimental diets containing 290 and 640 g/kg SBM (300 and 100 g/kg FM respectively). In another trial with 1 g gilthead sea bream fed the same diets, the same authors obtained inferior performance to the fish fed the control diet, but the fish fed the 450 g/kg diet still gave the best results.

Robaina *et al.* (1995) obtained slightly better results with 40 g gilthead sea bream fed diets containing 100 and 200 g/kg SBM (690 and 610 g/kg FM respectively) than fish fed a control diet containing no SBM and 770 g/kg FM, which in turn gave better performance than fish fed a 300 g SBM, 540 g/kg FM diet. The best performance was obtained with fish fed the 100 g/kg SBM diet. With smaller gilthead sea bream of initial weight 6.2 g, Nengas *et al.* (1996) obtained equal results when they fed the fish a diet containing 740 g/kg white FM, 0 g/kg SBM and a diet containing 240 g/kg SBM and 560 g/kg FM. A 120 g/kg SBM diet used in this trial gave a lower fish performance than both of these diets as did fish fed a diet containing 480 g/kg SBM (440 g/kg FM).

A number of other experiments with the gilthead sea bream have been carried out in which the amount of FM in the diet was reduced and substituted with other ingredients. These experiments show that the FM content in the gilthead sea bream diet can be reduced to quite an extent without any loss in performance. Experiments were carried out by Amaral (1994) and Bekkevold (1994) in which 2.3 and 11 g gilthead sea bream respectively were fed diets in which FM was gradually reduced from 670 to 0 g/kg using combinations of wheat, SBM, hydrolyzed feather meal and blood meal. The latter author did not find any significant differences in the performance of fish fed diets containing decreasing inclusions of FM (from 400 to 200 g/kg) compared to the control 670 g/kg FM diet, but Amaral (1994) obtained a decrease in performance as FM content was reduced from 200 to 0 g/kg with high mortality occurring with the fish fed the FM-free diet. In this latter work, the fish fed the 200 g/kg SBM diet gave an inferior performance to fish fed the control 670 g/kg FM diet. Davies *et al.*

(1993) fed 5 g gilthead sea bream a number of diets in which various meat and bone meals made up to 400 g/kg of the diet (470 g/kg white FM) but did not obtain any differences in performances when compared to fish fed the control 740 g/kg FM diet.

The results obtained in this experiment are not the first to have shown this negative relationship between SBM level in the diet and performance, and has been observed by numerous other authors in various species of fish. These include the work of Reinitz (1980; rainbow trout, *O. mykiss*), Alexis (1990; rainbow trout, *O. mykiss*), Pongmaneerat and Watanabe (1992; rainbow trout, *O. mykiss*), Watanabe *et al.* (1992; yellowtail, *Seriola quinqueradiata*), Webster *et al.* (1992b; blue catfish, *I. furcatus*), El-Sayed (1994; silver sea bream, *Rhabdosargus sarba*) and Davis *et al.* (1995; red drum, *Sciaenops ocellatus*).

On the other hand, numerous authors have recorded improved or at least equal performance in one parameter or more in fish fed diets in which SBM replaced FM, such as in the work of Davies *et al.* (1989; *Oreochromis mossambicus*), Webster *et al.* (1992a; blue catfish, *I. furcatus*), Reigh and Ellis (1992; red drum, *S. ocellatus*), Viyakarn *et al.* (1992; yellowtail, *S. quinqueradiata*), Shimeno *et al.* (1993; yellowtail, *S. quinqueradiata*,), Watanabe and Pongmaneerat (1993; rainbow trout, *O. mykiss*), Gallagher (1994; hybrid striped bass, *Morone saxatilis* x *M. chrysops*), Kaushik *et al.* (1995; rainbow trout, *O. mykiss*), Olli and Krogdahl (1995; Atlantic salmon, *Salmo salar*) and Stickney *et al.* (1996; rainbow trout, *O. mykiss*).

The three diets being considered here can be divided into two, diets 220C and 320C which only differed significantly in ANLU and EE, and fish fed diet 440C. Fish fed diet 220C ate slightly less than fish fed diet 320C and both ate significantly more than fish fed diet 440C. Although consumption of diet 320C was more than the consumption of diet 220C, fish fed diet 320C gave an inferior performance to fish fed diet 220C. Other authors have also noticed a reduction in food consumption when they fed fish diets containing increasing levels of SBM in at least one of their experimental diets (Balogun and Ologhobo, 1989; Reigh and Ellis, 1992; Davis *et al.*, 1995; Kubitza *et al.*, 1997) but others have even reported increases in consumption when SBM substituted FM in fish diets (Viyakarn *et al.*, 1992; Watanabe *et al.*, 1992; Watanabe and Pongmaneerat, 1993).

The main preoccupations with using SBM to replace FM in fish diets is the essential amino acid balance of this ingredient and the presence of antinutritional factors. There were no major differences between the three diets 220C, 320C and 440C in this experiment in terms of nutritional composition, although crude protein level and gross energy content decreased slightly as the level of SBM in the diet increased. The lipid and carbohydrate levels were very similar in these three diets. The first limiting essential amino acid is methionine in all diets. The amino acid contents do not follow any regular trend going from the low to the high SBM containing diets.

The trypsin inhibitor activities (TIA) of the three diets being considered were all below 0.3 mg/g diet which is below the level found by a number of authors to have an effect on growth and feed performance. Rumsey *et al.* (1993) suggested that TIA levels below 5 mg/g had little effect on rainbow trout, *O. mykiss*, Wilson and Poe (1985) suggested a maximum level of 3.2 mg/g for channel catfish, *I. punctatus*, Webster *et al.* (1992b) recommended a value of below 3.2 mg/g for blue catfish, *I. furcatus* and Wee and Shu (1989) recommended a maximum of 1.6 mg/g for Nile tilapia, *O. niloticus*.

The protein solubility of the SBM used in this experiment was 79.4% a value which lies between the 73 and 86% range in which Araba and Dale (1990) obtained the best growth in their trials with chicks. Protein solubility was used by these authors to assess the degree of heating, with values below this range indicating a possible overprocessing and reduction of amino acid (such as lysine and arginine) digestibility and availability.

The phosphorus contents of the three diets 220C, 320C and 440C were similar (although it decreased slightly going from diet 220C to 440C), but the decreasing phosphorus apparent digestibility coefficients (ADCs) of the fish fed these three diets clearly indicates that something was drastically affecting the digestibility of phosphorus in fish fed diet 440C compared to fish fed the other two diets. It might have been expected that a sequential decrease be obtained for phosphorus ADCs considering the increasing proportion of phytate in the diets as the SBM level in the diet increased.

With increasing SBM level in the diet the oligosaccharide level was also expected to increase, and therefore so too the antinutritional effects associated with it. Oligosaccharides are strongly suspected to cause an osmotic effect leading to fluid retention and a reduced passage time and thereby reducing digestibility (Reddy *et al.*, 1984; Wiggins, 1984; Huisman and Le Guen, 1991; Veldman *et al.*, 1993). Additionally, the oligosaccharides can be metabolized by the microflora in the intestine allowing increased microbial growth which also utilize nutrients to the detriment of the host (Champ *et al.*, 1990; Veldman *et al.*, 1993). The former of these effects is generally assessed by measuring the intestinal dry matter content. An increased oligosaccharide presence would result in an increased moisture content of the intestinal contents, i.e., less dry matter. The measurements taken for dry matter content in Experiment 1 are not clear. While there was a lower dry matter content in the intestines of fish fed diet 440C than fish fed either diet 220C or 320C, it would have been expected that fish fed diet 320C had intestinal dry matter contents lower than did fish fed diet 220C.

The effect on digestibility is even more difficult to assess. It is impossible to distinguish between fish digestion and microbial digestion which may be taking place in the digestive tract of the fish. The digestibilities of the different components of the diets indicates that there were little differences in protein, lipid or energy digestibilities. This is somewhat surprising considering the differences in performance obtained by the fish. Two factors could have explain this result. First of all, the digestive physiology could have become adapted to the feed intake so as to get as much out of the ingested food as possible. The second reason could lie in the activity of the bacteria in the gut, increased by the higher oligosaccharide content of the diet, consuming more of the nutrients in the gut and therefore contributing to the observed ADC values. Evidence of bacterial fermentation (by the production of volatile fatty acids) has been obtained by a number of authors (Rimmer and Wiebe, 1987; Lesel, 1993; Clements *et al.*, 1994; Kandel *et al.*, 1994; Kihara *et al.*, 1995; Smith *et al.*, 1996). Genovese *et al.* (1992) did not find any changes in intestinal microflora when fish were fed various diets containing SBM as compared to fish fed a SBM-free diet. On the other hand, Shivokene and Tryapshene (1985), Lesel (1988) and Lesel *et al.* (1988) found that the bacterial content of the intestine did vary with variation in diet composition. Wade *et al.* (1991) found that when humans were given soybean oligosaccharide extract (containing stachyose and raffinose) there was an increase in the intestinal microflora. However, to make matters even more complicated, Kitamikado *et al.* (1993) found that a number of oligosaccharide preparations actually had antibacterial activities.

A review of results obtained by numerous authors for fish performance, carcass composition, condition factor, hepatosomatic index and digestibilities clearly shows that there is a large amount of variability in the effect of SBM on these parameters. The results obtained in this experiment have been found to agree with some of these authors but not with others. This indicates that more work is required in order to understand better what factors are actually determining the observed results in terms of SBM inclusion.

The effect of supplementary enzymes

The addition of the two enzyme mixes to the 320 g/kg SBM diet (diets 320L and 320H) both gave a very good improvement in performance of the fish compared to fish fed the unsupplemented diet 320C. In the case of fish fed diet 320L, containing supplemental low pH protease and α-gal, this improvement was significant in all parameters studied except FCR. Fish fed diet 320H, containing high pH protease and α-gal, also gave a significantly higher SGR, ANPU, ANLU and EE than did fish fed diet 320C. Although the FCRs of diets 320L and 320H were not found to be significantly different from that of fish fed diet 320C, their FCRs were only 77 and 87% respectively that of fish fed diet 320C.

Feeding these two diets not only gave a better performance than fish fed the unsupplemented diet 320C, but also a better performance than fish fed the higher FM, lower SBM containing diet 220C. All parameters studied were better than those of fish fed diet 220C, diet 320L giving significantly better SGR, PER, ANPU, ANLU and EE than fish fed diet 220C, and diet 320H significantly higher SGR, ANLU, EE. Fish fed these two diets had improved FCR, although not shown to be significant, with fish fed diet 320L showing an FCR 87% that of fish fed diet 220C, but fish fed diet 320H being only slightly better. The same improvement in performance was not seen to such an extent in fish fed diets 440L and 440H. Fish fed diet 440L appeared to show an improvement in SGR, ANPU, ANLU and EE over

fish fed the unsupplemented diet 440C, but an inferior FCR and PER. Fish fed diet 440H gave the lowest performance in all parameters, and shall be discussed further below.

Fish fed diets 320L and 320H consumed almost the same quantity of food but more than fish fed the unsupplemented diets 220C and 320C. The reason why the fish fed diets 320L and 320H ate more food than fish fed either 220C or 320C is unclear when considered in terms of SBM content relative to FM content alone. This means that additional to the nutritional improvement brought about by the enzymes, there is also an increase in palatability (an effect of supplementary enzymes also seen by Hesselman *et al.* (1982), Maugle *et al.* (1983), Hesselman and Aman (1986), Cave *et al.* (1990) and Carter *et al.* (1994)). While small differences in the formulations used could somehow explain part of the differences in consumption between fish fed diets 220C and fish fed diets 320C, 320L and 320H, they obviously do not explain any differences between the three diets 320C, 320L and 320H. This means that either the carrier containing the enzyme had an effect or else there was some sort of enzyme activity even before the food was given to the fish, or just on impact with the water, possibly increasing the amount of attractants which might influence palatability and subsequent consumption. However, it is not possible to determine this with the available data but would be interesting to investigate further.

An increased level of consumption presents the fish with a higher amount of energy over the metabolic requirement available for growth. If more energy is available in the diet, protein sparing might occur. That this energy was made available is seen in the higher carcass lipid content of diets 320L and 320H over fish fed diets 220C and 320C, the former having the highest carcass lipid content and energy content of all fish used in this experiment. However, fish fed diet 320H did not have as high a carcass energy content as did fish fed diet 220C. The better energy utilization of fish fed diets 320L and 320H compared to fish fed diets 220C and 320C, is shown in the better ANLU and EE, and that protein sparing occurred compared to fish fed diets 220C and 320C is evident in the higher PERs and ANPUs.

The fish fed diets 320L and 320H showed higher protein, lipid, organic matter and energy ADCs than fish fed diets 220C and 320C. That there was still a lower carbohydrate digestibility in fish fed diets 320L and 320H, but improved over that of fish fed diet 320C, indicates that the enzymes were not completely successful in eliminating the effects of additional SBM on carbohydrate digestibility. The addition of these enzymes also improved the phosphorus utilization of the fish fed diets 320L and 320H over those fed diets 220C and 320C, a result which would have important environmental implications.

The enzymes used in this experiment had different pH optima (Feord, Pers. Comm., 1996). The low pH protease had an optimum activity around pH 3.0 and maintained up to 50% of this activity in the range 1.6 to 4.0. The high pH protease had its optimum pH around 8.5, with up to 50% of this activity maintained between pHs 7.2 and 10.2. The α-gal had its optimum at 5.0 and maintained up to 50% of this activity between 3.4 and 6.8. This means that both the low pH protease and the α-gal were active in the stomach of the gilthead sea bream where the pH ranged from 2.5 to 5.5 (Deguara, 1998). The α-gal would also have been active in the rest of the intestine, albeit with reduced activity. The high pH protease was not active in the stomach but was active in the rest of the intestine, although probably with a reduced activity throughout since the pH in the intestine ranged from 6.5 to 7.9 within the first 24 hours of feeding.

These pH optima and activity distribution could partly explain the better performance for all parameters of the low pH protease mixture compared to the high pH protease mixture. Since the pH of the stomach was closer to the optimum of the low pH protease than that of the intestine for the high pH protease, this enzyme would have been able to catalyze protein digestion to a higher degree than the high pH protease would. The α-gal would have been more active in the stomach. In addition to the actual breakdown of proteins by the proteases, the proteases would also indirectly assist in the breakdown of other components of the food, including other proteins, by making the food more accessible to the action of other enzymes, including the α-gal itself. But, while the α-gal could benefit by the action of the low pH protease in the stomach, it could not benefit so effectively from the activity of the high pH protease in the rest of the intestine. The specific mode of activity of the proteases in feed is unknown (Feord, Pers. Comm., 1997).

The intestinal dry matter contents of fish fed diets 320L and 320H were significantly lower than that found in fish fed diet 320C, and both lower than that found in fish fed diet 220C. If it is accepted that

oligosaccharides cause a higher osmotic pressure and hence cause the intestinal contents to carry more water, it would be expected that while diet 320C should have a lower intestinal dry matter content relative to diet 220C as a result of a higher oligosaccharide content, in view of the expected activities of the α-gal, fish fed diets 320L and 320H should have a higher dry matter content relative to fish fed diet 320C if not diet 220C as well.

The results shown by fish fed the 440 g/kg SBM diets 440C, 440L and 440H do not follow the same trends seen in fish fed diets 320L and 320H relative to fish fed diet 320C. Fish fed diet 440L consumed significantly more than fish fed diet 440C, an effect also seen for fish fed diets 320L and 320H. Although growth was higher than fish fed diet 440C, the food was not utilized so well, or at least not very more efficiently than fish fed diet 440C. In fact the FCR was higher, and the PER slightly lower. However, these fish managed to put down more protein than fish fed diet 440C and although the PER was slightly lower than that of fish fed diet 440C, the ANPU was slightly higher. The fish fed diet 440L also showed a slightly higher ANLU and EE than fish fed diet 440C although the carcass lipid contents were very similar. Fish fed diet 440L showed lower ADC values for all the nutritional components analysed, except for carbohydrate, than fish fed diet 440C (and indeed of all diets). The small rise in carbohydrate ADC could have been due to the activity of the α-gal, but why there was a drop in protein, lipid, organic matter, energy and phosphorus ADCs is unclear, although these would help to explain how the fish fed diet 440L had a higher FCR and lower PER than fish fed diet 440C.

It is difficult to understand the effect of the additional enzymes on the performance obtained, small as it is. Theoretically one of two effects would have been expected. First, the higher level of SBM in the diets (440 g/kg) could have given more scope, so to speak, for the enzymes to bring about a positive effect compared to the unsupplemented diet. The second possibility is that the positive effect of the enzymes was masked by the negative effects of the higher SBM level present, i.e., the amount of enzyme present was not sufficient to bring about an effect with 440 g/kg SBM as it had been with the 320 g/kg SBM diets. As for fish fed diet 440H, although they still consumed almost as much as fish fed diet 440C, the performance is greatly below what would be expected. Assuming there were no effect of the enzymes, it would be expected that a performance similar to that of fish fed diet 440C would be obtained. Diet 440H was the only diet to which liquid enzymes had been added instead of dry enzymes. Why this should have had such a negative effect is also not known. The increased water as a result of addition of the liquid would not have had an effect and the moisture content of the diet was similar to that of diet 440C. What remains is the reduced food intake due to reduced palatability, again for an unknown reason, since liquid enzymes have been used frequently in experiments without such an adverse effect being noted.

The few experiments that have been carried out on the use of supplementary enzymes in juvenile fish diets have shown some positive results when compared to fish fed unsupplemented diets. Carter *et al.* (1994) fed 95 g Atlantic salmon (*S. salar*) a diet containing 340 g/kg SBM, 440 g/kg FM to which a cocktail of proteases (trypsin (porcine); alkaline protease (*Bacillus* sp.); acid protease (*Rhizopterus* sp.)) and carbohydrases (amylase (malt), amylase (bacterial) and cellulase) had been added at 1 g/kg. The SGR and FCR of fish fed the enzyme supplemented diet were superior (1.08%/day and 0.76 respectively) to fish fed the unsupplemented SBM diet (0.66%/day and 1.13 respectively) and even better than that of fish fed the control 660 g/kg FM diet (0.89%/day and 0.81 respectively). Bogut *et al.* (1995) fed 46 g carp, *Cyprinus carpio*, a 360 g/kg protein diet to which 0.5, 1.0 and 1.5 g/kg of an enzyme cocktail (Polyzyme) containing amylase, protease, α-glucanase, α-glucosidase and cellulase. The fish fed the 1.5 g/kg Polyzyme diet had significantly better growth and feed utilization.

However, with 38 g rainbow trout, *O. mykiss*, Cardenete *et al.* (1993) found no differences in performance between fish fed a basal diet of which 40% of the protein was provided by cotton seed meal and to which a commercial enzyme cocktail (Kemzyme Dry) was added at 0.4, 1.2 and 3.6 g/kg. Common carp, *C. carpio*, fry of 0.5 g fed a basal diet to which 1.9 and 5.7 g/kg bovine trypsin had been added showed only slight improvements in food utilization over fish fed the basal diet, with the fish fed the higher trypsin containing diet giving the best results (Dabrowski and Glogowski (1977). FCR, PER and ANPU improved from 1.74, 1.34 and 19.6% to 1.71, 1.40 and 20.4% respectively.

Finnfeeds International have carried out a number of trials using commercial enzyme cocktails. In one trial carried out by Finnfeeds (1996c) on 4 g Nile tilapia, *O. niloticus*, the addition of 1 g/kg

Pescazyme 5602 (which also contained a protease) to a diet containing 630 g/kg SBM, 0 g/kg FM, values of 3.16%/day for SGR and 1.47 for FCR were obtained in fish fed this diet which was not only superior to the performance of fish fed the unsupplemented diet (with values of 2.78%/day and 1.84 respectively) but also of the fish fed a 470 g/kg SBM, 120 g/kg FM diet (with values of 2.94%day and 1.70 respectively). With 5 g common carp, *C. carpio*, fed a basal diet containing 505 g/kg SBM, 0 g/kg FM to which the same enzyme (at 1 g/kg) had been added, the improvement over the unsupplemented diet was also obtained with an improvement in SGR from 2.08 to 2.38%/day and FCR from 1.66 to 1.40, but the performance of these fish was still below that of fish fed a 410 g/kg SBM, 100 g/kg FM control (with an SGR of 2.52%/day and FCR of 1.32) (Finnfeeds, 1996d). In another trial, this time with 4 g mirror carp, *C. carpio*, an enzyme mix of protease and carbohydrase was included at 1 g/kg to the diet (Feord, Pers. Comm., 1996). In this experiment, both the fish fed the 490 g/kg SBM, 50 g/kg FM diet and the supplemented diet performed better than fish fed the 250 g/kg FM, 200 g/kg SBM control diet. However, addition of enzymes still slightly improved the SGR of the fish fed the supplemented diet from 1.58 to 1.61%/day and the FCR from 1.97 to 1.94.

A number of trials have also been carried out on the use of enzymes to reduce the effect of antinutritional factors. In fish this has been limited to the use of phytases to act against phytic acid. Cain and Garling (1995) fed 2 and 15 g rainbow trout, *O. mykiss*, diets containing 310 g/kg SBM which had or had not been pretreated with phytase. In both sizes of fish used, the weight gain of the fish increased, from 646 to 757% and from 273 to 375% respectively, FCR decreased from 0.93 to 0.86 and from 1.15 to 0.85 respectively. Protein utilization of the larger fish increased from 27 to 39% when fish were fed the phytase treated SBM diet. Phosphorus discharged in the faeces was decreased in both sizes of fish fed the pretreated SBM diet compared to the fish fed the untreated SBM diet from 2.55 to 1.88 g/kg diet fed and from 1.73 to 0.34 g/kg diet fed respectively. Feeding phytase supplemented diets (500 to 2000 units/kg) to 6.5 g catfish, *I. punctatus*, increased growth from 51 g/fish for the fish fed the unsupplemented diet to up to 63 g/fish and reduced FCR from 1.99 down to a minimum value of 1.70 (Robinson *et al.*, 1996). Rodehutscord and Pfeffer (1995) fed 8.7 and 29 g rainbow trout, *O. mykiss*, a diet containing 630 g/kg of soybean products and to which phytase (*Aspergillus niger*, 1000 Units/kg) had been added. Addition of phytase increased the growth of fish compared to the fish fed the unsupplemented diets, of the smaller fish from 12.1 to 18.1 g/fish and the larger fish from 5 to 5.5 g/fish. FCRs were also improved when fish were fed the supplemented diet from 1.41 to 1.25 and from 3.02 to 2.67 in the small and larger fish respectively. Phosphorus utilization of the fish fed the phytase supplemented diet was increased, from 17 to 49% and from 6 to 25% for the two sizes of fish respectively. Schaefer *et al.* (1995) fed 40 g common carp, *C. carpio* a 530 g/kg SBM, 150 g/kg FM diet to which two levels of *A. niger* phytase had been added (500 and 1000 units/kg). The average weight gain of the fish fed the unsupplemented diet was 108 g/fish, which increased to 136 and 147 g/fish as the level of phytase was increased. FCRs of the fish decreased from 1.4 to 1.2 to 1.1 and ANPU increased from 18.2 to 23.2 to 24.8% as phytase level in the diet increased. The phosphorus utilization increased from 30% in the fish fed the unsupplemented diet to 42 and 47% when fish were fed the two supplemented diets respectively.

Although positive effects of enzyme supplementation on growth, feed utilization and digestibilities have been observed, these improvements were not always consistent or could be accounted for, and in the experiments where various inclusion levels of enzymes had been used improvements were not always correlated with enzymes dosage. The volume of literature available on the use of enzymes in fish diets is in fact limited, but promising, and more work is required to give a better understanding of how the enzymes are working and on the factors affecting their activity.

Conclusions

Due to constraints on both supply and cost, it would seem inevitable that, in the near future, FM levels in feeds for intensively cultured species such as gilthead sea bream will fall. This will result in an increase in the level of plant proteins in fish feeds and it is obvious that considerable effort needs to be directed at improving the utilization of these ingredients. The numerous trials carried out by the poultry and pig industry, and a number of those carried out on aquaculture species, show that use of supplementary enzymes does have the potential to alleviate the increasing FM supply problem for an ever expanding industry. The practical implications of some of these results on a commercial scale would be significant. First of all, the price of diets containing less FM should be lower than diets of higher FM content. Additionally, the superior performance obtained would mean that fish are brought

to market sooner using a smaller quantity of food. Another beneficial effect would be a lower environmental impact.

This experiment has shown that dietary enzyme supplementation can have a positive effect on the performance of the gilthead sea bream and in some cases significantly so. This positive effect has been seen to not only alleviate the negative effect of replacing dietary FM with SBM but also to bring about a superior performance compared to fish fed a higher FM containing diet. However, the same effect was not seen in fish fed the highest SBM containing diets and further research into the impact of enzyme inclusion levels should be carried out.

This work only considered two ingredients, SBM and FM, and only three types of enzymes. Although the enzymes were found to have an effect on performance of fish, the reasons underlying how these effects were brought about (or not) is still unknown, and is therefore an area where further research is obviously required. Additional experiments and trials need to be carried out, to confirm the results obtained in this experiment and to increase the understanding of how the enzymes act, before definite conclusions and methods of application can be made.

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