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SUCCESSFUL REPLACEMENT OF FISHMEAL BY PLANT PROTEINS IN DIETS FOR THE GILTHEAD SEABREAM, *SPARUS AURATA* L.

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Abstract

Soy protein concentrate, wheat gluten, and corn gluten meal were evaluated in combination and as sole dietary protein sources in diets for gilthead seabream. A growth trial and digestibility determinations demonstrated the effectiveness of these plant proteins as alternatives to fishmeal. Digestibility trials indicated superior protein digestibility for soy protein (92%), wheat gluten (96%), and corn gluten (90%) in comparison with fishmeal (86%), while energy digestibility was higher than fishmeal (84%) only in wheat gluten (91%; it was 75% for soy protein and 72% for corn gluten). For the growth trial, eight isonitrogenous and isoenergetic (as-fed basis) diets were formulated with differences in the protein sources. Growth in the range of 40-130 g was superior to the fishmeal control with the diet containing wheat gluten but inferior in the diets containing soy protein concentrate or corn gluten meal. Diets that replaced 25-100% of the fishmeal with a mixture containing equal portions of all three plant proteins outperformed the control with a 9-16% greater weight gain and 4-10% lower feed conversion ratio. The use of soy protein or corn gluten as the sole protein source in diets for seabream is not recommended but their use in combination with wheat gluten can provide a partial or complete alternative to fishmeal. However, the cost of supplemental arginine made replacement economic at only the lowest replacement level (25%). An in-depth evaluation of the need for this amino acid in the protein mixture could significantly affect the feasibility of using higher replacement levels.

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Introduction

Due to the limited and fluctuating worldwide supply of fishmeal, its ever increasing use in animal production, and the resulting rise in its cost, the search for alternative sources of protein in aquaculture diets has been underway for a number of years. Much of this search centers on plant-based proteins due to their greater abundance and potentially lower costs. The protein content of plant ingredients is lower than that of fishmeal, making replacement of fishmeal on an equal protein basis complicated. On the other hand, the use of protein concentrates manufactured from plant ingredients allows almost equal replacement. However, many protein concentrates require amino acid and/or mineral supplementation to meet the nutritional requirements of the fish.

Concentrates of soy protein, corn gluten meal, and wheat gluten have been researched as possible fishmeal replacements in aquaculture diets. Success has been achieved in partially replacing fishmeal by concentrates as the sole protein source, in most cases with amino acid or other nutrient supplementation, in aquacultured Atlantic halibut (*Hippoglossus hippoglossus*; Berge et al., 1999), Atlantic salmon (*Salmo salar*; Storebakken et al., 1998, 2000; Refstie et al., 2001), European sea bass (*Dicentrarchus labrax*; Ballestrazzi et al., 1994; Tibaldi and Tulli, 1998), Japanese flounder (*Paralichthys olivaceus*; Kikuchi 1999), rainbow trout (*Oncorhynchus mykiss*; Alexis et al., 1985; Hardy, 1996; Stickney et al., 1996), turbot (*Scophthalmus maximus*; Day and Plascencia-Gonzalez, 2000), and yellowtail (*Seriola quinqueradiata*; Takii et al., 1990). Complete substitution of fishmeal with soy protein or wheat gluten has been achieved only in rainbow trout (Kaushik et al., 1995; Rodehutscord et al., 1995).

The use of combinations of plant based ingredients as a fishmeal substitute seems promising as nutrient deficiencies, primarily amino acids, in one ingredient may be supplied by a second feed component if the diet is properly formulated. In a few studies with such mixtures, partial (Gomes et al., 1995) to almost complete replacement (Kaushik et al., 2004) of fishmeal was achieved with rainbow trout and European sea bass, respectively. Fishmeal was completely replaced by plant protein mixtures in rainbow trout diets with part of the dietary protein supplied by animal sources (Watanabe et al., 1997).

Fishmeal replacement studies with the gilthead seabream (*Sparus aurata*), a mainstay of Mediterranean aquaculture, have had limited success. Kissil et al. (2000) reported that, at a 30% replacement level of fishmeal (27% of fishmeal protein) with soy protein, there were signs of growth reduction in seabream while Robaina et al. (1997) reported that corn gluten meal successfully replaced 30% of fishmeal protein. No studies on the substitution of fishmeal by wheat gluten have been published for seabream but unpublished data from our laboratory indicate that wheat gluten, when properly supplemented with limiting amino acids, can replace fishmeal in seabream diets.

Wheat gluten is an effective binder in some aquaculture feeds but limited industrial production (600,000 tons in 2002; Popineau et al., 2003) makes greater use of wheat gluten in aquaculture feeds impractical. Kaushik et al. (2004) recently suggested that the use of wheat gluten in combination with other plant proteins may be economically feasible as a fishmeal substitute for European sea bass. The objective of this preliminary study was to determine to what extent a mixture of soy protein, corn gluten, and wheat gluten could replace fishmeal in gilthead seabream diets.

Materials and Methods

Diet preparation. Seven experimental diets were formulated to contain 45% protein and 20% lipid using soy protein concentrate (Danpro A, Central Soya European Proteins A/S, Denmark), wheat gluten (Amygluten 160, Amylum Group, France), or corn gluten meal (Matmor Inc., Central Feed Mill, Israel) as the sole source of protein or as part of an equal protein mixture of the three (Table 1). The mixture was substituted in the diet for 25, 50, 75 or 100% of the fishmeal. An eighth diet, the

Table 1. Formulation and proximate composition of eight experimental diets used in a growth trial of gilthead seabream.

	Diet							
					Fishmeal:plant protein mixture			
	Fishmeal	*Wheat gluten*	*Soy protein*	*Corn gluten*	*75:25*	*50:50*	*25:75*	*0:100*
Ingredient (g/kg fed)								
Fishmeal	680	-	-	-	475	300	155	-
Wheat gluten	-	510	-	-	60	110	150	190
Soy protein concentrate	-	-	700	-	65	120	160	210
Corn gluten meal	-	-	-	650	65	120	160	210
Wheat meal [a]	200	110	24	33	173	138	121	90
Fish oil [b]	110	190	195	180	135	155	170	180
DL-Methionine [c]	-	6.5	6	3	2	3	4	5
L-Lysine HCl [d]	-	21	-	21	-	4	9	15
L-Threonine [e]	-	5	-	5.3	-	-	-	3
L-Arginine HCl [f]	-	13.2	-	13	-	3	6	7
Vitamin premix [g]	10	10	10	10	10	10	10	10
Mineral premix [h]	-	25	15	25	5	12	15	25
Dicalcium phosphate	-	60	50	60	10	25	40	55
Sipernat [i]	-	50	-	-	-	-	-	-
Cost of ingredients [j]								
Protein	$0.46	$1.99	$0.80	$1.55	$0.50	$0.82	$1.14	$1.30
Arginine cost	-	$1.23	-	$1.21	-	$0.28	$0.56	$0.65

Table 1. Continued

Analysis (per kg)								
Dry matter (g)	923	919	925	912	920	924	922	931
Crude protein (g)	448	451	439	458	440	449	445	453
Crude lipid (g)	217	200	204	196	201	210	197	202
Ash (g)	124	107	99	76	103	95	88	88
Methionine (g) [k]	12.6	12.2	12.5	12.6	13	12.7	12.5	12.3
Lysine (g) [k]	36.1	26.6	28.5	27.1	29.2	27.2	26.9	27.6
Threonine (g) [k]	19.2	15	18.2	16.7	17.6	16.1	14.6	16.1
Arginine (g) [k]	26.3	26.6	34.7	25.4	24.4	25.7	26.9	26.4
Phosphorus (g)	20.4	11.5	14	14.3	17.1	15.3	14.3	13
Gross energy (MJ)	22.2	22.8	22.0	23.5	21.8	21.9	22.3	23.0
Digestible energy (MJ) [l]	18.7	21.2	18.2	18.9	18.4	18.6	19.0	19.5
Digestible protein (g) [l]	373	431	402	413	377	397	402	419

[a] Dry matter 87%, gross energy 16.5 MJ/kg (apparent digestibility 65%); crude protein 11.8% (apparent digestibility 76%).
[b] Gross energy 38.5 MJ/kg (apparent digestibility 95%).
[c] Rhodia, France; feed grade.
[d] Eurolysine, France; feed grade.
[e] Kyowa Hakko Kogyo Co. Japan; feed grade (98%).
[f] Applichem GmbH, Germany; chemically pure.
[g] Vitamins (per kg diet): A 12,000 IU; D_3 1900 IU; E 150 mg; thiamine 20 mg; riboflavin 30 mg; niacin 50 mg; Ca-pantothenate 30 mg; pyridoxine 5 mg; folic acid 8 mg; K 5 mg; inositol 250 mg; B_{12} 0.12 mg; biotin 0.25 mg; C 250 mg; choline chloride 3 g.
[h] Mineral premix (per 10 g premix): KCl 3.6 g; MgO 833 mg; MnO 73.5 mg; ZnO 75 mg; $FeCO_3$ 275 mg; KI 1.8 mg; $CuCO_3$ 40 mg; $CoCO_3$ 0.66 mg; Na_2SeO_3 0.4 mg.
[i] Degussa, Germany; SiO_2 (98%) used as diet filler.
[j] Calculated according to costs in Israel in January 2004.
[k] Calculated.
[l] Calculated assuming additivity as ADCs of individual ingredients (Lupatsch et al., 1997).

control, contained fishmeal (Matmor Inc., Central Feed Mill, Israel) as the only source of protein. To avoid perceived amino acid deficiencies, the amino acid compositions of the experimental diets were compared with that of fishmeal and amino acids were supplemented where necessary. Amino acid contents of the feed ingredients were taken from the literature. Dicalcium phosphate was supplemented to satisfy the phosphorus requirement determined for seabream at our laboratory (unpubl. data).

The diets were ground in a hammer mill using a 2 mm screen and analyzed for proximate composition. Samples were analyzed in duplicate with a maximum variation of 2.5% from the mean being acceptable. Dry matter was calculated from weight loss after drying at 105°C for 24 h. Crude protein was measured using the Kjeldahl technique by multiplying N by 6.25. Crude lipid was measured gravimetrically after 5 min homogenization of the sample in chloroform-methanol (2:1), separation, and vacuum drying (Folch et al., 1957). Ash content was calculated from weight loss after incineration of samples in a muffle furnace for 24 h at 550°C. Phosphorus content was determined after ashing, using the vanado-molybdate method (AOAC, 1980). Gross energy of samples was determined by combustion in a Parr bomb calorimeter using benzoic acid as the standard. The chromic oxide content of feed and fecal samples used in the digestibility trial was determined with a modification of the method of Furukawa and Tsukahara (1966).

Digestibility of the feed ingredients was determined according to the procedure of Lupatsch et al. (1997). Digestible protein and energy contents were calculated from the apparent digestibility coefficients determined for all the ingredients (Tables 1 and 2). Apparent digestibility coefficients were determined for protein and energy from duplicate groups of gilthead seabream (300-400 g). Chromic oxide (8 g/kg) was used as the inert marker and feces were collected by stripping. Diets were mixed thoroughly and steam pelleted in a California laboratory pellet mill and stored at -20°C until used.

Fish and experimental design. Fish were obtained from a commercial farm in the Gulf of Aqaba and, after grading, were randomly stocked at 25 per tank in 250 l conical fiberglass tanks at an average weight of 40.8±1.7 g. The tanks were supplied with filtered seawater (41 ppt) from an open system at 22-24°C and at an exchange rate of 8 l/min for the 85-day growth trial. The fish were fed a commercial seabream diet (Matmor, Israel) before stocking and switched to their respective experimental diets within 24 h after transfer. Triplicate groups of fish were fed each diet at a fixed ration twice per day with care taken that no food was left uneaten. The ration was adjusted after weighing every 14 days. The ration size was taken from feeding tables developed at the National Center for Mariculture for seabream based on their protein and energy requirements for growth (Lupatsch and Kissil, 2003).

An initial sample of 10 fish was taken for body composition at the start of the trial. Samples of 10 fish from each experimental tank were taken at the end. Samples were stored frozen and, before analysis, cut into small pieces and ground twice through a meat grinder with a 3-mm die. Samples were taken to determine dry matter content (24 h at 105°C) and the remaining homogenate was oven dried. The dried samples were subsequently mixed in a kitchen blender before proximate analysis according to the above procedures.

Calculations. Feed conversion ratio (FCR) was calculated using the expression: FCR = feed consumed (as fed basis)/fish weight gain (wet wt). Digestible protein and energy intake per fish were calculated using the average feed eaten/fish x digestible protein or energy value calculated for each experimental diet. The utilization of dietary protein and energy was assessed as the retention of these nutrients relative to the amounts of digestible protein or energy consumed by the fish, i.e., productive protein value (PPV) = protein gain x 100/digestible protein consumed; energy retention value (ERV) = energy gain x 100/digestible energy consumed.

The estimated cost of the protein in the ingredients was calculated based on ingredi-

Table 2. Proximate composition and apparent digestibility coefficients (ADC) of protein sources used in the experimental diets.

	Fishmeal	*Wheat gluten*	*Soy protein*	*Corn gluten*
Composition (per kg as fed)				
Dry matter (g)	920	930	925	923
Crude protein (g)	628	778	623	628
Lipid (g)	134	8.0	4.8	19.8
Ash (g)	179	11.6	59.4	15.5
Crude carbohydrate (g) *	-	132.4	237.8	259.7
Phosphorus (g)	28.7	1.8	7.6	3.0
Gross energy (MJ)	19.2	21.0	18.5	21.1
ADC (%)				
Protein	86	96	92	90
Energy	84	91	75	72

* Calculated as crude carbohydrate = dry matter - crude protein - lipid - ash.

ent costs in Israel in January 2004. The contribution of the supplemental arginine to this cost, the one ingredient not economically produced, appears separately.

Statistical analysis. Analysis of variance and the Duncan multiple-range test were used to detect significant differences (p<0.05) among treatments in final body composition, weight gain, FCR, feed intake, PPV, and ERV (the last two parameters after Arcsine transformation). Statistical analyses were carried out with SPSS for Windows (5.0).

Results

The composition and protein and energy digestibility of the protein sources used in the experimental diets appear in Table 2. The protein in all three plant sources was more available to the seabream than that of the fishmeal, with digestible energy highest in wheat gluten, then fishmeal, soy protein concentrate and corn gluten. Although the diets were formulated to be isonitrogenous and isoenergetic on an ‘as fed’ basis, differences in protein and energy digestibility of the protein source resulted in differences in availability of these nutrients among the diets.

Average weight after 85 days increased 256-310% with five of the diets growing significantly better than the control (278.8%; Table 3). Amongst the four protein sources, digestible energy and protein intakes were highest for wheat gluten, as expected from the higher energy and protein digestibility of this ingredient. In general, protein retention was highest in the better growing treatments, i.e., the wheat gluten, mixed protein, and control diets, and lowest in the two diets that fostered the poorest growth, i.e., the soy and corn gluten diets. Energy retention was more complicated, with the wheat gluten diet having the highest retention (significantly higher than the control) and the four mixed protein diets with retention values between the wheat gluten and control diets. As with protein retention, the poorest performing diets, corn gluten and

Table 3. Growth performance parameters per fish (means of triplicate groups of 25 fish each).

Diet	*Initial wt (g)*	*Final wt (g)*	*Wt gain (g)*	*FCR*[1]	*Digestible protein intake (g)*	*PPV*[2]	*Digestible energy intake (kJ)*	*ERV*[3]
Fishmeal	41.57	115.9	74.3^{b}	1.34^{b}	37.16^{e}	34.7^{a}	$1.86^{b,c}$	35.7^{b}
Wheat gluten	41.16	126.2	85.1^{a}	1.22^{a}	44.56^{a}	34.5^{a}	2.19^{a}	41.6^{a}
Soy protein	41.69	108	66.3^{c}	1.45^{c}	$38.65^{d,e}$	29.6^{b}	$1.75^{c,d}$	29.2^{c}
Corn gluten	39.15	100.4	61.2^{c}	1.44^{c}	36.37^{e}	29.1^{b}	1.66^{d}	30.4^{c}
75:25	42.51	124.3	81.8^{a}	$1.29^{a,b}$	$39.82^{c,d}$	35.4^{a}	1.94^{b}	37.7^{b}
50:50	41.15	127.2	86.1^{a}	1.21^{a}	$41.4^{b,c}$	34.9^{a}	1.93^{b}	36.8^{b}
25:75	39.85	123.9	84^{a}	1.21^{a}	$40.9^{b,c,d}$	35.5^{a}	1.93^{b}	$38.7^{a,b}$
0:100	41.01	122	81^{a}	$1.26^{a,b}$	$42.57^{a,b}$	34.8^{a}	1.98^{b}	36.6^{b}

Values in a column with the same superscript do not significantly differ ($p>0.05$).
1 Feed conversion ratio = feed consumed (as fed basis)/fish weight gain (wet wt).
2 Productive protein value = protein gain x 100/digestible protein consumed.
3 Energy retention value = energy gain x 100/digestible energy consumed.

soy, had significantly lower energy retention than the rest.

The diet containing only wheat gluten and the mixed protein diets promoted 9-15.9% better growth than the control fishmeal diet (Table 4). Significantly poorer performance compared to the control was obtained for the corn gluten (-17.5%) and soy protein (-10.8%) diets. FCR followed a pattern that was close to weight gain but only the wheat gluten, 50:50, and 25:75 diets were significantly better than the control, out-performing it by 9-9.7%.

Changes in body composition in the growth trial appear in Table 5. In general, dry matter, lipid content, and gross energy increased while protein, ash, and phosphorus showed little change. Differences in body composition did not significantly differ at the end of the trial.

Discussion

Fish diets, like other animal feeds, are made from a variety of feed ingredients to satisfy the nutrient requirements of the fish. Feed ingredients are incorporated according to the nutrients they provide so that the diet meets all nutritional needs. The likelihood that a number of ingredients together will meet the needs of the fish is greater than that a single ingredient will satisfy them. It is on this basis that a mixture of plant proteins was chosen as a potential substitute for fishmeal in gilthead seabream diets.

Protein concentrates of soy, corn gluten, and wheat gluten have previously been used in attempts to completely replace fishmeal in aquaculture feeds. Individually, only soy protein and wheat gluten were able to completely replace fishmeal in rainbow trout diets (Kaushik et al., 1995; Rodehutscord et al., 1995). In the present study, wheat gluten and the combination of all three concentrates not only successfully replaced fishmeal in gilthead seabream diets but also produced superior growth and feed conversion ratio. This may be related to the higher digestible protein and energy intake of the fish due to the higher availability of protein and energy in wheat gluten. The calculated costs of the protein-providing ingredients in these diets suggest that the protein mixture was economically feasible only at the 25% replacement level. At this level, the cost of replacing the fishmeal would raise the cost of the protein component in the feed by less than 10% whereas the addition of arginine to the other four diets (wheat gluten, 50:50, 25:75, and 0:100) increased the cost of the protein ingredients very significantly, by 78-332%.

Table 4. Improvement in growth and feed efficiency of the diets that performed better than the control, compared to the control. Numbers appearing in bold are statistically different from the control diet.

Diet	*ΔWt gain (%)*[1]	*ΔFCR (%)*[2]
Fishmeal (control)	-	-
Wheat gluten	**14.5**	**9**
75:25	**10.1**	3.7
50:50	**15.9**	**9.7**
25:75	**13.1**	**9.7**
0:100	**9.0**	6

[1] Δweight gain (%) = $[(\text{Wt gain}_{treatment} - \text{Wt gain}_{control})/\text{Wt gain}_{control}] \times 100$
[2] ΔFeed conversion ratio (%) = $[(FCR_{control} - FCR_{treatment})/FCR_{control}] \times 100$

Table 5. Whole body proximate composition (g/100 g wet wt) and gross energy contents (MJ/kg wet wt) of fish at the start and finish of the feeding trial.

	Initial (n = 10)	*Final (means of three samples of 10 fish each (±SD)*							
		Fishmeal	*Wheat gluten*	*Soy protein*	*Corn gluten*	*75:25*	*50:50*	*25:75*	*0:100*
Dry matter	30.35	33.4±1.2	34.8±0.5	33.0±1.1	33.2±1.2	34.0±0.6	33.2±1.2	34.0±0.8	34.0±0.8
Protein	15.78	16.8±0.4	17.3±0.3	16.7±0.5	16.7±0.3	16.7±0.1	16.5±0.7	16.8±0.7	17.5±0.6
Lipid	9.77	12.4±1.3	13.3±0.6	12.3±0.3	12.7±0.8	13.3±0.6	13.2±0.7	13.6±0.1	13.1±0.9
Energy	7.9	8.9±0.5	9.5±0.3	8.8±0.5	8.9±0.5	9.3±0.2	9.0±0.4	9.3±0.3	9.2±0.3
Ash	3.9	3.8±0.1	3.9±0.1	4.0±0.2	4.1±0.1	3.7±0.1	3.6±0.2	3.8±0.3	3.8±0.2
Phosphorus	0.71	0.70±0.01	0.68±0.01	0.74±0.04	0.75±0.01	0.67±0.0	0.65±0.01	0.69±0.09	0.71±0.04

Values among treatments did not significantly differ ($p>0.05$).

The synthetic amino acids used in the diets brought their amino acid configuration close to that of fishmeal. Further experimentation will determine whether the quantity of some of the amino acids could be reduced. Manipulation of the contribution of each protein source to the mixture may enable reduction of supplementation without adverse effects on the growth of the fish. This is especially important in the case of arginine, an amino acid that is not yet mass-produced economically enough to allow use as a feed ingredient on a commercial scale.

Differences in protein digestibility among the four proteins used in this study suggest an advantage to the plant proteins over that of the fishmeal. This may be the result of the amount of industrial processing the plant proteins underwent in comparison to the fishmeal or of the quality of the fishmeal chosen for the trial. The intention of this trial was to use fishmeal that is commonly used in commercial seabream feeds, therefore it was obtained from a local feed mill that produces seabream feed.

The differences in digestible energy among the three plant proteins are probably related to their relative carbohydrate content. Soy protein and corn gluten have almost double the amount of crude carbohydrate found in wheat gluten. Carbohydrate is less available as an energy source to more carnivorous fishes (Dabrowski and Guderley, 2002) such as seabream, which feed mainly on crustacea and other shellfish in nature. The greater energy available from wheat gluten compared to fishmeal is probably due to the use of part of its protein as an energy source in these fish.

The lack of significant differences in body content of lipid and energy among the treatments is in contrast to results reported for European sea bass by Kaushik et al. (2004) who reported a significant increase of both in sea bass raised on diets containing increasing levels of a plant protein mixture instead of fishmeal. The increase was thought to be the result of increased lipogenesis with increased fishmeal replacement. In our study, seabream fed diets with wheat gluten or graded levels of the protein mixture had slightly elevated lipid and, consequently, energy levels than those fed the control fishmeal diet, but this may have been the result of their larger size. Lupatsch et al. (1998) showed that seabream deposit more fat with increasing size, associated with a reduction in moisture content.

The poor performance of soy protein and corn gluten as sole protein sources, even when supplemented with essential amino acids needed to raise their levels to those of fishmeal, suggests other nutritional deficiencies or anti-nutritional factors in soy protein and corn gluten. The effects of either of these are likely to be more apparent over a longer growth trial. Soy proteins contain a lectin (soybean agglutinin) that binds to intestinal epithelial cells in salmonids, causing pathological changes and affecting nutrient absorption when present at high levels in the feed (Buttle et al., 2001). In addition, protease inhibitors in soybeans and corn gluten reduce the activity of proteolytic enzymes in seabream, soybean inhibitors having a stronger effect than those in corn gluten (Moyano et al., 1999). Other anti-nutritional factors have been reported in both protein sources (Dong et al., 2000) that could have contributed to a reduction in their effectiveness for growth.

This study demonstrates the successful substitution of a plant protein mixture for fishmeal in diets for gilthead seabream. Although the mixture in its present form is more expensive than the current cost of fishmeal, the increase in growth and feed efficiency that it promotes partially reduces the cost difference. More accurate information on the limiting amino acids of the protein sources in the mixture could significantly lower the cost of the protein mixture in commercial seabream feeds. Further investigation is needed to improve the economic feasibility of using this protein mixture as a partial or complete substitute for fishmeal in seabream feeds.

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