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Carob seed germ meal as a partial substitute in gilthead sea bream (*Sparus aurata*) diets: Amino acid retention, digestibility, gut and liver histology

Silvia Martínez-Llorens, Rosa Baeza-Ariño, Silvia Nogales-Mérida, Miguel Jover-Cerdá, Ana Tomás-Vidal *

Research Group of Aquaculture and Biodiversity, Institute of Animal Science and Technology, Polytechnic University of Valencia, Camino de Vera, 14. 46071-Valencia, Spain

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ABSTRACT

The use of carob seed germ meal (CG) as a substitute for fish meal was evaluated in fingerlings (average weight 10 g) of gilthead sea bream fed isonitrogenous (46% Crude Protein, CP) and isolipidic (19.5% Crude Lipid, CL) diets containing four CG levels (0, 17, 34 and 52%). The duration of the trial was 83 days. The diets were tested in triplicate, and the fish were fed to satiation twice daily. The apparent protein and energy digestibility coefficients decreased in response to the dietary inclusion of CG. The decreases in the values of the ADC ranged from 93% for the 0 diet to 80% for the 52 diet. The amount of digestible Arg in the diets increased with the inclusion of CG, from 3.2 g Arg 100 g^{-1} in the 0 diet to 4.81 g Arg 100 g^{-1} in the 52 diet. The digestible Arg content increased in the diets because the amounts of the remaining digestible amino acids decreased.

The fish reached weights of 72, 72, 69 and 53 g on the 0, 17, 34 and 52 diets, respectively. The 52 diet gave the lowest specific growth rate (SGR, 1.95% day^{-1}) relative to those found for the other three diets (2.32% day^{-1}). The 52 diet produced the least satisfactory results for feed intake (FI) and the food conversion ratio (FCR) (2.23 g 100 g $fish^{-1}$ day^{-1} and 1.38, respectively). The efficiency of retention of His, Phe and Leu in the fish fed the 52 diet showed the lowest values (17.3, 25.5 and 28.5%, respectively), but the efficiency of retention of Met in the fish fed the 52 diet showed the highest value (48.2%).

In the distal intestine and liver, histological alterations were found in the fish fed the 34 and the 52 diets. In fish fed the 52 diet, the mucosal fold was significantly shorter and thinner and exhibited a smaller number of goblet cells. These changes could affect nutrient uptake by modifying the digestibility of the diet. The histology of the liver did not show severe degradation in any treatment.

The results of the present experiment demonstrated that CG can be included at levels up to 34% in diets for short term feeding of gilthead sea bream without any adverse effects on fish growth and nutritive parameters.

1. Introduction

The prices of all of the ingredients used in feeds for aquaculture, especially fish meal and fish oil, have risen rapidly in recent years. The cost of feeds has increased in parallel with the cost of these ingredients. As a consequence of these developments, the aquaculture industry is trying to become more economically sustainable by focusing on improved feeding techniques.

Gilthead sea bream (*Sparus aurata* L.) is a species of great importance. However, its production in Spain decreased 14.1% from 2009 to 2010 (APROMAR, 2011). The main cause of this reduction is the decrease in the price of gilthead sea bream. This price decrease has made the farming of gilthead sea bream less profitable.

To improve the profitability of fish production and to reduce the cost of feeds, a great variety of alternative and sustainable ingredients are under investigation as potential replacements for fish meal. A new diet must ensure the welfare of the fish because a healthy digestive system is fundamental for ideal performance. Ingredients based on vegetable crops are the most promising alternative feed ingredients, particularly if they are produced by terrestrial agriculture in the region and could contribute directly to the sustainability and cost-effectiveness of fish farming (Adamidou et al., 2011). Vegetable protein meals with high protein content have been the most widely tested as substitutes for fish meal. Leguminous seeds (Pereira and Oliva-Teles, 2002), particularly soybeans, can be used either in extracted form (Bonaldo et al., 2008; Martínez-Llorens et al., 2007, 2009) or in full-fat form thanks to their adequate amino acid profile (Alexis, 1990). The ideal diet would have an EAA (Essential Amino Acids) profile similar to the amino acid composition of the fish (Akiyama et al., 1997). Information about the amino acid contents of the fish is an indispensable tool for determining the dietary AA (Amino Acids) requirements. An alternative protein

* Corresponding author. Tel.: +34 96 3879752; fax: +34 96 3877439.
E-mail address: atomasv@dca.upv.es (A. Tomás-Vidal).

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meal with an adequate amino acid profile would guarantee successful growth in fish fed this diet. Nevertheless, the dietary level of vegetal protein is critically related to decreases in protein digestibility (Santigosa et al., 2008) and to equal AA digestibility (Sánchez-Lozano et al., 2009, 2011).

The nutritional potential for certain agricultural byproducts has not been adequately studied. This situation limits their utilisation as animal food ingredients. The high prices paid for the protein concentrates used for feeding in aquaculture and the shortages of these concentrates have stimulated studies of the nutritional and economic feasibility of byproducts and wastes as sources of protein. It therefore appears that the resources offered by such products need to be further exploited and thoroughly evaluated nutritionally.

Carob seed germ meal (CG) is a byproduct obtained from the germ of the carob seed (*Ceratonia siliqua*) after the separation of the gums and the fibrous coating of the seed. In the dry state as feed, CG provides approximately 95.5% dry matter and has a high protein content (45–50% CP), a biological value similar to soybean meal. CG is, however, considerably less expensive than soybean meal (34% less in 2009, FAOSTAT, 2009a,b). Carob seed germ meal is therefore of potential economic interest. The world production of carob was estimated at approximately 181 000 t/year (FAOSTAT, 2009a,b). Spain leads the world in carob production, with an average of 65 000 t/year, followed by Italy, Portugal, Morocco, Greece, Cyprus, Turkey, Algeria and other countries.

Some studies have investigated the use of carob seed meal as a substitute for fish meal. Alexis et al. (1985) tested diets with 20 and 35% of CG in fingerling rainbow trout and found that the resulting performance was low. Alexis (1990) evaluated diets with three levels of CG inclusion (22, 42 and 58%) and showed that the growth of the rainbow trout decreased as the level of the plant byproduct in the diet increased. Diets containing CG resulted in low growth, a reduction in body protein and an increase in liver protein. These results indicated possible amino acid deficiencies and imbalances at high inclusion levels. The most important antinutritional factors reported for carob seeds are tannins (Nachtomi and Alumot, 1963). The main antinutritional characteristics of tannins are related to their ability to reduce protein utilisation and digestibility. Tannins can form complexes with proteins, binding to hydrophobic regions by hydrogen and ionic bonds, which can cause deficiencies and imbalance that are detrimental for fish growth (Alexis, 1990).

The aim of the present study was to evaluate the effects of CG in diets for gilthead sea bream on growth, nutritive parameters, digestibility, and amino acid retention and to investigate possible morphological alterations in the distal intestine and liver of the fish.

2. Materials and methods

2.1. Diets

Four extruded isolipidic (19.5% CL) and isonitrogenous (46% CP) diets were formulated with different dietary levels of carob seed germ meal (CG, CAROB, S.L., Mallorca, Spain) (0, 17, 34 and 52%). Hereafter, the four diets will be identified by their CG levels. The formulation and the composition of the diets are given in Table 1.

The diets were prepared with the cooking extrusion process, using a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). The processing conditions were as follows: a screw speed of 100 rpm, a temperature of 110 °C, and a pressure of 40–50 atm. The experimental diets were assayed in triplicate groups. The fish were fed by hand twice a day (9.00 and 16.00) until apparent satiation. The pellets were distributed slowly to allow all fish to eat. The uneaten diet was collected and dried to determine feed intake (FI).

2.2. Growth trial and fish sampling

Gilthead sea bream (*S. aurata*) fingerlings were obtained from a local fish farm (Piscimar, S.L., Castellón, Spain) and transported alive to the Fish Nutrition Laboratory of the Polytechnic University of Valencia, Spain. Prior to the feeding trial, all fish were acclimated to the indoor rearing conditions for 2 weeks and fed a standard sea bream diet (48% crude protein, CP; 23% crude lipid, CL; 11% Ash; 2.2% crude fibre, CF; and 14% nitrogen free-extract, NFE). Groups of 54 fish (average weight 10 g) were housed in 12 cylindrical fibreglass tanks (three per treatment). The capacity of each tank was 1750 l.

The duration of the trial was 83 d. The trial was conducted in a recirculating marine water system (65 m^3 capacity) with a rotary mechanical filter and a gravity biofilter (approximately 6 m^3). The water temperature ranged from 22.5 ± 2.8 °C (mean ± SD). The salinity was 33 ± 1 g l^{-1}. The level of dissolved oxygen was 6.5 ± 0.5 mg l^{-1}. The pH ranged from 7.5 to 8 during the trial.

All tanks were equipped with aeration. The water was heated by a heat pump installed in the system. The photoperiod was natural in both trials, and all tanks had similar light conditions. All fish were weighed at approximately 30-day intervals. Prior to weighing, the fish were anaesthetised with 30 mg l^{-1} of clove oil (Guinama®, Valencia, Spain) containing 87% of eugenol. At the end of the growth trial, all fish were individually weighed. Five fish were randomly sampled from each tank and used for the determination of biometric parameters and for proximate analysis. The samples from each tank were pooled and stored at −30 °C. Three guts and livers per tank were collected for histological analysis.

3. Proximate composition and amino acid analysis

Chemical analyses of the dietary ingredients were performed prior to diet formulation. Diets and their ingredients, as well as the whole fish, were analysed according to AOAC (1990) procedures: dry matter

Table 1
Formulation and proximate composition of the experimental diets.

	Diets			
	0	17	34	52
Ingredients (g kg^{-1})—international feed number in parentheses				
Fish meal herring (5-02-000)	600	492	384	270
Carob seed meal		169	339	518
Wheat (4-05-268)	199	135	71	3
Maltodextrin (4-08-023)	50	50	50	50
Soybean oil (4-07-983)	40	40	40	40
Fish oil (4-08-048)	101	104	106	109
Multivitamin and minerals mix[v]	10	10	10	10
Proximate composition (% dry weight)				
Dry matter	91.54	91.99	92.17	92.16
Crude protein (% CP)	45.78	45.88	46.14	46.05
Crude lipid (% CL)	19.70	19.86	19.94	19.29
Ash (%)	10.15	9.35	8.48	7.96
Crude fibre (% CF)	0.37	0.93	1.49	2.07
NFE (%)[w]	24.00	23.98	23.95	24.63
DP (%)[x]	42.78	43.06	40.59	40.52
DL (%)[y]	19.31	19.66	19.34	17.36
DE (MJ/kg^{-1})[z]	21.01	21.20	19.12	18.43

[v] Vitamin and mineral mix (values are g kg^{-1} except those in parenthesis): Premix: 25; Choline, 10; DL-a-tocopherol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1 000 000 IU kg^{-1}; calcipherol, 500 IU kg^{-1}; DL-a-tocopherol, 10;menadione sodium bisulphite, 0.8; thiamine hydrochloride, 2.3;riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamine, 25;nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07;ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides 12.
[w] Nitrogen free-extract, NFE (%) = 100-%CP-%CL-%Ash-%CF.
[x] Digestible protein (DP) was calculated based on the respective values of apparent digestibility coefficients (Protein $ADC_{protein}$, %): diet 0 = 93; diet 17 = 94; diet 34 = 88; diet 52 = 88.
[y] Digestible lipid (DL) was calculated based on the respective values of apparent digestibility coefficients (Lipid ADC_{lipid}, %): diet 0 = 98; diet 17 = 99; diet 34 = 97; diet 52 = 90.
[z] Digestible energy (DE) was calculated based on the respective values of apparent digestibility coefficients (Energy ADC_{energy}, %): diet 0 = 91; diet 17 = 92; diet 34 = 82.; diet 52 = 8. Energy: Calculated using: 23.9 kJ g^{-1} proteins, 39.8 kJ g^{-1} lipids and 17.6 kJ g^{-1} carbohydrates.