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Meat and bone meal as partial replacement of fishmeal in diets for gilthead sea bream (*Sparus aurata*) juveniles: Diets digestibility, digestive function, and microbiota modulation

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ABSTRACT

A digestibility trial was conducted to evaluate the effect of fishmeal (FM) replacement with meat and bone meal on diets digestibility, digestive enzymes activity, and microbiota modulation in gilthead seabream (*Sparus aurata*) juveniles fed diets (45% CP; 20% CL) including 0, 50, and 75% of protein from MBM (diets MBM0; MBM50; MBM75).

The ADC of protein was high and unaffected by dietary MBM level, whereas the ADC of energy was higher with diet MBM50 than with MBM0. The ADC of essential amino acids was also high and not affected by diet composition, except for the ADC of phenylalanine and tyrosine, which were lower in diet MBM75 than in the other diets. Pepsin, trypsin, chymotrypsin, lipase, and total alkaline proteases activities were all unaffected by diet composition. Dietary inclusion of MBM modulated gastrointestinal tract microbiota, decreasing the average number of operational taxonomic units and microbial richness. Dietary MBM inclusion promoted an increase of *Vibrio*, *Bacillus*, and *Mycobacterium* genera, whereas colonization by *Staphylococcus* and *Corynebacterium* decreased. Overall, present results indicate that up to 75% of FM protein can be replaced by MBM protein in diets for gilthead seabream juveniles without major adverse effects on diet digestibility and digestive function. However, gastrointestinal microbiota was modulated, and further research should be conducted to evaluate the impact of gastrointestinal microbiota modulation on immune and health status of gilthead seabream.

1. Introduction

In intensive production, fish meal (FM) has been the preferred protein source for carnivorous fish due to its high protein content, balanced amino acid profile, high digestibility and palatability, and lack of anti-nutritional factors (Gatlin et al., 2007; Tacon et al., 2011). Worldwide, aquaculture remains the largest consumer of FM (Karapanagiotidis, 2014) but production constraints and supply fluctuations have increased the search for alternative protein sources. Thus, sustainable growth of carnivorous fish aquaculture industry will depend on increased use of alternative feed resources.

One of the most economically important species in Mediterranean aquaculture is gilthead seabream (*Sparus aurata*) (Basurco et al., 2011; Oliva-Teles et al., 2011) but overproduction in the last decades has led to a decreasing in its sale price (Flos et al., 2002). Production of gilthead seabream is still heavily dependent on high quality FM for diets production (Karapanagiotidis, 2014). Since feeding can account for almost half of the overall variable costs in Mediterranean intensive aquaculture (Martinez-Llorens et al., 2012), replacing FM with more cost-effective protein sources without compromising growth, quality, and welfare of farmed fish, would greatly increase profitability by reducing feeding costs (Martinez-Llorens et al., 2012; Perez-Jimenez et al., 2012).

Plant feedstuffs have been widely used to replace FM in aquafeeds due to their wider availability and competitive costs (Gatlin et al., 2007; Oliva-Teles et al., 2015). However, except for some particular pant protein concentrated, the use of plant ingredients by carnivorous species may be limited due to the high carbohydrate content, lack of

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balanced amino acid profile, and presence of anti-nutritional factors (Oliva-Teles et al., 2015). Indeed, results often fall short of the desired and only rarely high substitution levels have been reached without producing detrimental effects (Oliva-Teles et al., 2015). Poorer digestibility is also a cause of concern when using alternative protein sources in fish diets (Magalhães et al., 2015; Wei et al., 2015). The use of plant feedstuffs in aquaculture also competes in the international market with that of farm animal production, biofuel production, and direct use for human consumption (Karapanagiotidis, 2014). Under this scenario, underutilized protein sources from terrestrial animal origin may provide a beneficial, sustainable and cost-effective alternative to reduce the constraints imposed by the limited availability of FM.

Processed animal proteins (PAP) are defined as “animal protein derived entirely from Category 3 materials, which have been specially treated so as to render them suitable for direct use as feed material or for any other use in feedstuffs” (EU Commission Regulation EC/142/2011). In 2001, the EU restricted the use of these ingredients for feeds, including aquafeeds (EU Commission Regulation EC No. 999/2000), due to the arise of transmissible bovine spongiform encephalopathy (BSE) disease in ruminants. As a result, the potential of PAP for feeding European fish species was neglected from that moment onwards. However, the ban of PAP's use was partially lifted in 2013, allowing the use of PAP derived from non-ruminant animals as feedstuffs for aquaculture species, but maintaining the prohibition of intra-species recycling of protein (EU Commission Regulation, EC No. 56/2013). This opens a completely new range of feedstuffs that can be used for FM replacement in European aquaculture.

One of these PAPs is meat and bone meal (MBM) of non-ruminant origin, manufactured all across Europe, with an average production of 3.5 million tons/year in the EU (Coutand et al., 2008). MBM are protein rich ingredients, with balanced amino acid profile, highly digestible and palatable, and lacking anti-nutritional factors (Suloma et al., 2013). Earlier studies have demonstrated the potential using MBM in aquafeeds for several farmed species worldwide (El-Sayed, 1998; Bureau et al., 2000; Bharadwaj et al., 2002; Tidwell et al., 2005; Ai et al., 2006; Wei et al., 2006; Goda et al., 2007; Li et al., 2010; Kader et al., 2011; Lee et al., 2012) including gilthead sea bream (Davies et al., 1993; Robaina et al., 1997). However, results on their efficacy as feed ingredients have been inconsistent. Fish species specificities, including different feeding habits and, particularly, differences in the nutritional quality of MBM, could be in the basis to this discrepancy in results. Indeed, nutritional quality of MBM is highly dependent on the raw materials and processing techniques used to produce it (Kureshy et al., 2000; Wang et al., 2010). Moreover, the harmful effect of excessive heat applied to MBM may be even more pronounced in the EU due to the revised legislation of technological processing of PAP (EC No. 1069/2009; temperature over 133 °C, pressure, 3 bar by steam for 20 min; maximum particle size, 50 mm), and may compromise the bioavailability of MBM protein and amino acids. High ash content, due to the presence of bones and other inorganic matter, can also compromise MBM digestibly and may limit its use in fish diets, by decreasing nutrients' bioavailability (Bureau et al., 1999; Robaina et al., 1997).

Fish gastrointestinal tract (GIT) microbiota is a complex ecosystem, composed by a wide diversity of microorganisms and having different functions, including maintenance of intestinal integrity, protection against invasive pathogens, and contributing for host nutrition (de Medina et al., 2013). GIT microbiota can be significantly modulated by numerous factors including age, genetics, and feeding of the animals (Estruch et al., 2015). Still, when compared to farm animals and humans, this understanding is relatively limited in fish (Clements et al., 2014). GIT microbiota modulation by dietary inclusion of plant ingredients has been previously studied for several farmed species worldwide (Heikkinen et al., 2006; Refstie et al., 2006; Ringo et al., 2006a, 2006b; Estruch et al., 2015). However, GIT microbiota modulation by dietary inclusion of MBM is yet to be assed and, to date only one study evaluated terrestrial animal by-products effects in fish microbiota (Hartviksen et al., 2014b). Given the interaction between host, microbiota and diet, understanding these relationships is crucial for the development and evaluation of novel feedstuffs while maximizing fish health and welfare.

Recently, we have shown that up to 50% of FM protein could be replaced by MBM protein in diets for gilthead seabream juveniles without compromising growth performance and feed utilization (Moutinho et al., 2017). The aim of this work is to provide further insights on the effects of FM replacement by MBM for gilthead sea bream juveniles by evaluating diets digestibility, digestive enzymes activity and GIT microbiota modulation.

2. Materials and methods

2.1. Diet composition

Three isoproteic (45% crude protein) and isolipidic (20% crude lipid) diets were formulated with FM protein replaced by MBM protein at increasing levels: 0 (control diet, MBM0), 50% (MBM50) and 75% (MBM75). Diets were prepared by cooking-extrusion using a semi-industrial twin-screw extruder (CLEXTRAL BC-45; Firmity, St. Etienne, France), at 100 rpm speed screw, 110 °C temperature, and 40–50 atm, to form 2–3 mm diameter pellets. Then diets were stored at − 30 °C until the beginning of the experiment. Ingredients and chemical composition of the experimental diets are presented in Table 1 and the amino acid composition in Table 2.

2.2. Growth trial and rearing system

The growth trial was not object of the present study and is detailed described elsewhere (Moutinho et al., 2017). In brief, gilthead sea bream (*Sparus aurata*) juveniles were supplied by a local fish farm (Piscimar, S.L., Castellón, Spain) and transported to the Fish Nutrition Laboratory of the Polytechnic University of Valencia. Fish were adapted to the indoor rearing conditions for two weeks while fed a standard commercial diet (48% CP; 23% CL). The growth trial was performed in

Table 1
Ingredients and chemical composition of the experimental diets.

	MBM0	MBM50	MBM75
Ingredients (g kg^{-1} DM)			
Fish meal[a]	574	287	143
Wheat meal[b]	263	176	132
Meat and bone meal[c]	–	409	615
Soy oil	94	33	3
Fish oil	49	74	87
Vitamin and minerals mix[d]	20	20	20
Chemical composition (% DM)			
Dry matter (DM, %)	90.0	91.9	91.5
Crude protein (CP)	44.0	43.8	45.3
Crude lipid (CL)	21.4	19.0	20.6
Crude fiber (CF)	2.20	1.84	1.66
Ash	10.3	18.8	20.1
Energy (kJ g^{-1})	20.3	18.8	19.8
NFE (%)[e]	22.1	16.6	12.3

[a] Fish meal (93.2% DM, 70.7% CP, 8.9% CL, 15.1% Ash, 19.7 kJ^{-1} Energy); Vicens I Batllori S.L., Girona, Spain.
[b] Wheat meal (92.4% DM, 17.1% CP, 2.4% CL, 78.3% CHO, 2.4% Ash); Piensos Y Cereales Desco, Museros, Valencia, Spain.
[c] Meat and bone meal (97.0% DM, 53.1% CP, 15.3% CL, 26.9% Ash, 17.7 kJ^{-1} Energy); VALGRA S.A., Beniparrell, Valencia, Spain.
[d] Vitamin and mineral mix (g kg^{-1}): 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.
[e] Nitrogen-free extract, NFE (%) = 100 − %CP − %CL − %CF − %Ash.