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Review

Effect of vegetable based diets on growth, intestinal morphology, activity of intestinal enzymes and haematological stress indicators in meagre (*Argyrosomus regius*)

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ABSTRACT

A performance trial (88 days) was undertaken to evaluate the effects of replacing fishmeal (FM) and/or fish oil (FO) in a practical formulation for juvenile meagre with a mixture of plant proteins (PP) and vegetable oils (VO), in terms of growth performance, nutrient utilization, intestinal structure and functionality and several haematological stress indicators. Twelve homogenous groups of 75 meagre (mean initial body weight: 55.4 ± 3.5 g) were fed one of four isonitrogenous (51% DM), isolipidic (17% DM) and isoenergetic diets (22.9 MJ · kg^{-1} DM). A FMFO diet was formulated with high levels of marine protein sources (45%) and fish oil as the sole fat source. Based on this formulation, 60% of fish oil was replaced by a blend of vegetable oils (diet FMVO). Two other diets were formulated with a 50% replacement of marine proteins by plant protein sources. One of these plant protein-rich diets contained fish oil as the sole fat source (PPFO), while in the other 60% of fish oil was replaced by a blend of vegetable oils (PPVO). Growth of meagre was not significantly affected ($P > 0.05$) by the replacement of fishmeal and fish oil by ingredients of vegetable origin. Meagre fed the plant-protein rich diets (PPFO and PPVO) showed a significant enhancement ($P < 0.05$) of feed efficiency (FE) and retention of protein (PER), lipids and energy. Dietary lipid source had no effect ($P > 0.05$) on FE, PER and nutrient retention. Dietary treatments had no effect ($P > 0.05$) on the whole-body composition of fish. This good tolerance towards plant protein sources exhibited by meagre was further corroborated by the apparent digestibility data of individual ingredients. As expected, fish fed the blend of vegetable oils showed a reduction of total n-3 series fatty acids and a concomitant increase of total n-6 series fatty acids. Dietary changes on both protein and lipid sources did not affect ($P > 0.05$) intestinal morphology, activity of intestinal brush border enzymes and selected haematological stress indicators in fish. Signs of liver steatosis were found in meagre, but not directly associated to dietary changes of protein and lipid sources. Meagre showed a good ability to cope with vegetable based diets. However, although not statistically significant in this short-term trial, present data also suggests possible detrimental effects on fish performance associated to concomitant high inclusion levels of plant protein and vegetable oils.

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1. Introduction

Reared under an adequate water temperature profile, meagre (*Argyrosomus regius*) is one of the best candidate species for large scale production in the Mediterranean region due to its fast growth (over 700 g fish after 12 months and 2–2.5 kg after 24 months; Ribeiro et al., 2013), favourable farming under intensive conditions and a high market interest. Currently, meagre is essentially fed with commercial diets developed for gilthead seabream and European seabass, and dedicated scientific studies validating the nutritional needs of this new species are extremely scarce. Meagre is characterised as having carnivorous feeding habits, since previous studies have showed that juvenile meagre has a high dietary protein requirement (50%) (Chatzifotis et al., 2012) and only tolerates moderate dietary lipid levels (17%) (Chatzifotis et al., 2010). Recently, it was reported that in comparison to a control diet containing 30% fishmeal, the inclusion of plant-protein sources and a concomitant reduction of fishmeal to 20% of the formulation did not affect growth or feed utilization of meagre juveniles. However, a further reduction of fishmeal to a 10% level, significantly depressed the growth performance of the fish (Estévez et al., 2011). However, it is worth pointing out that despite being isoenergetic, the experimental diets of this latter study did not maintain isonitrogenous conditions, with crude protein levels varying from 46.7% in the fishmeal-rich diet and 42% on the diets containing higher levels of plant-protein sources. Under isonitrogenous (50% crude protein) and isolipidic conditions (17% crude fat), Velazco-Vargas et al. (2013) found that a 30% inclusion of defatted soybean meal was feasible in diets for meagre. Selection of ingredients for feed formulation requires data on the apparent digestibility coefficients (ADCs) of nutrients. Quantitative information on the digestive capacity of nutrients and energy from several feed ingredients are now being generated in juvenile meagre (Magalhães, 2013; Olim, 2012).

At high inclusion levels, vegetable ingredients have been reported to affect overall fish welfare and performance, in a species dependent manner (Montero and Izquierdo, 2011), due to nutritional imbalances and/or the presence of anti-nutritional factors (Francis et al., 2001, Merrifield et al., 2011). In a carnivorous species like Atlantic salmon, high replacement levels of fishmeal by soybean meal induced drastic structural alterations in the intestine, which evolve into a pathological situation named soybean-induced enteritis (Baeverfjord and Krogdahl, 1996). In addition, being an important barrier of the fishes immune system towards infections, disrupting gastrointestinal tract had drastic consequences on fish homeostasis since its protective function was impaired. These lesions lead to concomitant consequences on organism homeostasis with negative impacts on overall performance. Decreases in digestive enzymes activities and inhibition of adsorption of brush border enzymes, alterations on the intestinal morphology (shortening of the intestinal mucosa folds, supranuclear vacuoles in enterocytes, widening of lamina intestinal lesions), inflammation process, fat deposition in liver and enterocytes, loss of intestine selectivity, lower resistance to diseases were symptoms described for fish fed diets with high inclusions of soybean meal (Baeverfjord and Krogdahl, 1996; Gabrielsen and Austreng, 1998; Ganga et al., 2011; Hedrera et al., 2013; Krogdahl et al., 1999, 2003; Knudsen et al., 2008; Martínez-Llorens et al., 2012; Merrifield et al., 2011; Oxley et al., 2010; Uran, 2008). Studies with other species, like common carp and gilthead seabream reveal a similar pattern of soybean-induced enteritis but at much higher levels of inclusion when compared to Atlantic salmon (Uran, 2008), suggesting a different species susceptibility towards vegetal ingredients.

Successful replacement of both fishmeal and fish oil was achieved for some fish species that reduced or even annulled the negative impacts on growth and pathologies like soybean-induced enteritis (Martínez-Llorens et al, 2012; Sitjà-Bobadilla et al., 2005). Still, physiological and metabolic alterations were observed, being different among fish species that at long term might compromise fish welfare. Being a strict carnivorous species, meagre tends to display lower tolerance to vegetable ingredients. Therefore, it is important to assess other physiologic parameters beyond growth to have a global and integrated vision of fish performance. In this context, two trials were undertaken, one to determine the apparent digestibility of nutrients and energy of feed ingredients and experimental diets. Secondly, a performance trial was undertaken to evaluate the effects of replacing fishmeal and/or fish oil in a practical formulation for juvenile meagre with plant proteins and vegetable oils, in terms of growth performance, feed utilization, intestinal structure and functionality and several haematological stress indicators.

2. Material and methods

2.1. Apparent digestibility of feed ingredients

For assessing the digestibility of individual ingredients, the experimental design comprised a reference diet (REF) and test diets with 70% of the same mixture used for the Reference diet and 30% of each individual test ingredient, according to the methodology recommended by National Research Council (NRC) (2011). The basal mixture was fishmeal-rich and contained 52% crude protein, 17% lipid and 1% chromic oxide as an inert digestibility marker (Table 1). The REF diet consisted of 100% of the basal mixture, while test diets combined the basal mixture and each individual test ingredient in 70:30 ratio. Test ingredients were: solvent-extracted dehulled soybean meal (SBM), rapeseed meal (RSM), sunflower meal (SFM), corn gluten meal (CGM), soy protein concentrate (SPC), pea protein concentrate (PPC) and wheat gluten (WG). Diets were manufactured by extrusion in conditions identical to those described below for the growth trial diets. The proximate composition of the diets and test ingredients is presented in Table 1.

The digestibility measurements were performed at the Experimental Research Station of CCMAR (Faro, Portugal). The apparent digestibility coefficients (ADC) of experimental diets were determined in duplicate, by the indirect method with homogenous groups of 60 meagre (body weight: 170 grams) reared in circular tanks supplied with seawater (temperature 22 ± 3 °C, salinity 36 psu, dissolved oxygen > 6.0 mg/L). After 7 days of adaptation to the chromic oxide marked diets, faeces were collected during 10 consecutive days, through an outflow water decantation system. After daily collection, faeces were frozen at −20 °C. Pooled faeces from each group of fish were freeze-dried prior to analysis. ADC of the dietary nutrients and energy were calculated as follows:

$$\mathrm{ADC}\ (\%) = 100 \times \left[1 - \frac{\text{dietary } Cr_2O_3 \text{ level}}{\text{faecal } Cr_2O_3 \text{ level}} \times \frac{\text{faecal nutrient or energy level}}{\text{dietary nutrient or energy level}}\right]$$

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