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Vegetable re-esterified oils in diets for rainbow trout: Effects on fatty acid digestibility

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ABSTRACT

The present study aimed at determining the effect of re-esterified oils with different monoacylglycerol (MAG) content, produced from two different vegetable sources with different degrees of saturation, palm and rapeseed, on fatty acid digestibility in rainbow trout. Re-esterified oils were obtained from a chemical esterification process using acid oils (free fatty acids (FFA)-rich by-products from the refining of vegetable oils) and glycerol (by-product of biodiesel production). This process, which produces the formation of triacylglycerols (TAG), reduces the content of FFA present in the acid oil and generates a redistribution of the fatty acids in the glycerol molecule. This redistribution could increase the amount of saturated fatty acids (SFA) located at the sn-2 position of acylglycerols. Moreover, it allows obtaining fats with a certain proportion of MAG, known for being good emulsifiers. Therefore, a higher nutritive value of re-esterified oils than that of acid oils might be expected. A 21-day feeding trial where triplicate groups of rainbow trout were fed nine experimental diets formulated to contain a 21% of different experimental oils was carried out. For each source, four different types of oil were used: native, re-esterified low in MAG, re-esterified high in MAG and acid. A commercial fish oil was used for the control diet. Although re-esterified oils had better apparent digestibility coefficients (ADC) of SFA than their corresponding acid oil diets, no improvement in SFA digestibility was observed in rainbow trout fed re-esterified oils diets compared to those fed native oil diets, not even when a high content of MAG was present. Although this improvement did not occur, both palm and rapeseed re-esterified oils could be incorporated as a fat source in diets for rainbow trout without negatively affecting fatty acid digestibility values. The study concluded that fatty acid digestibility in the experimental oils was more affected by their degree of saturation than by their positional distribution and lipid class composition of the oils.

1. Introduction

Nowadays, aqua feeds account for about 75% of the global consumption of fish oil (FO). Its production depends on the availability of wild fisheries, which has decreased since the mid-1990s. In spite of the progressive drop-off in the use of FO by aquaculture, its global demand and price have been increasing due to both the rapid expansion of aquaculture sector and its growing use by the nutraceutical industry. Thus, the price of FO is expected to rise 70% from 2010 to 2030 (FAO, 2014). As a consequence, the use of vegetable oils (VO) as an alternative source of energy to replace FO in commercial fish feeds has increased. VO are renewable sources produced in large volumes that have a lower price than FO, being palm, soybean, rapeseed and sunflower the most produced (Gunstone, 2011). They are mainly used for food and feed, although its use as feedstock for energy production has increased steadily (Behr and Gomes, 2010; Jayasinghe and Hawboldt, 2012).

Refining is an industrial procedure necessary to render VO to an edible form that has bland flavour and odour, clear appearance, light colour, stability to oxidation and suitability for frying (Brooks et al., 2013; FAO, 1994). It removes compounds other than triacylglycerols (TAG) in order to obtain a TAG-rich oil (by 99%). Some of these other compounds are valuable and can be recovered for subsequent use (Nuchi et al., 2009). They can be used for the production of special feed "technical" lipids such as calcium soaps, hydrogenated lipids, re-esterified, and mono- and diglyceride oils which can satisfy specific nutritional requirements (Parini and Cantini, 2008). Acid oils are free fatty acids (FFA)-rich by-products generated from the refining process (Nuchi et al., 2009). They can be considered a cheaper alternative to the use of vegetable native oils. However, studies performed in broiler chickens (Blanch, 1996; Wiseman and Salvador, 1991) reported that acid oils have a lower energy value than that of native oils, which have

Abbreviations: ADC, apparent digestibility coefficient(s); DAG, diacylglycerol(s); FA, fatty acid(s); FFA, free fatty acid(s); MAG, monoacylglycerol(s); MUFA, monounsaturated fatty acid(s); NMR, nuclear magnetic resonance; PUFA, polyunsaturated fatty acid(s); SFA, saturated fatty acid(s); TAG, triacylglycerol(s); VO, vegetable oil(s).

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been related to a lower digestibility due to their high FFA content. The nutritive value of these oils might be improved by means of a chemical esterification process, which generates the formation of TAG and thus reduces the content of FFA. These TAG are formed after the reaction of FFA from vegetable acid oils with glycerol (Parini and Cantini, 2009), the latter being a by-product of biodiesel production. As this TAG synthesis is not selective, it can result in a different fatty acid positional distribution within the TAG molecule than their corresponding native oils. In native VO, SFA (palmitic and stearic) are mainly located in the external positions of the TAG molecules (sn-1 and sn-3) while the sn-2 position contains a high proportion of unsaturated fatty acids (oleic, linoleic and linolenic) (Hunter, 2001; Karupaiah and Sundram, 2007). During lipid digestion in mammals, pancreatic lipase hydrolyses the external positions of the TAG, being 2-monoacylglycerols (MAG) and FFA as the main products of the lipid digestion process. FFA that are mono- (MUFA) or polyunsaturated (PUFA) fatty acids will be mainly incorporated into micelles and absorbed. However, impaired digestibility is found for free long chain SFA due to its hydrophobicity, high melting point and the possibility to form insoluble soaps in the gut and thus be lost in faeces (Berry, 2009; Hunter, 2001; Small, 1991). The fatty acid located in sn-2 remains bound to the glycerol molecule as 2-MAG, which is directly absorbed (Schulthess et al., 1994). In fish, the predominant type and specificity of pancreatic lipase is still quite controversial and seems to vary greatly among species. Even so, a bile salt-dependent pancreatic lipase with sn-1,3-specific hydrolytic activity has been pointed as the main lipolytic enzyme in different species (Bogevik et al., 2007; Gjellesvik et al., 1992; Tocher, 2003). Thus, when a SFA is esterified in the sn-2 position, it may have a superior absorption, as it has been described in rats (Renaud et al., 1995), piglets (Innis et al., 1995), broiler chickens (Smink et al., 2008) and human infants (Kennedy et al., 1999). It could then be expected that the distribution of FA in chemically re-esterified oils resulted in a higher content of SFA in sn-2 position in the TAG and this could result in a higher digestibility of these oils compared to their native counterparts.

Another important difference between re-esterified and native oils could be the proportion of the different lipid classes – TAG, diacylglycerols (DAG) and MAG – that are present in the new re-esterified oil (Parini and Cantini, 2009). Chemical esterification process allows obtaining fats with the same fatty acid profile, but with different contents of TAG, DAG and MAG according to the process conditions (i.e. proportions of FFA and glycerol). As it has been pointed out, lipid digestion aims to reduce large lipid molecules (TAG and DAG) to smaller ones (MAG and FFA) for their absorption. Of these lipid classes, MAGs have been long known as good emulsifiers due to their amphiphilic nature and surface-active properties (Cruz-Hernandez et al., 2012; Hess et al., 1995; Martin et al., 2014), so digestibility values might improve when a major MAG content is present in the dietary fat.

To the best knowledge of the authors and to-date, there are no studies in the literature reporting the use of randomly re-esterified VO in fish diets. A higher nutritive value of re-esterified oils than of acid oils might be expected as a result of the reduction of the amount of FFA that takes place during esterification. Similarly, changes in their physicochemical properties compared to their corresponding native oils could be obtained due to both the higher amount of SFA in sn-2 and their higher proportion of MAG. Thus, the present study aims at determining the effect of re-esterified oils with different MAG contents, produced from palm and rapeseed acid oils, on fatty acid digestibility in rainbow trout (*Oncorhynchus mykiss*) as a first step to determine if they can be suitable fat sources for fish diets.

2. Materials and methods

2.1. Experimental diets

Nine experimental diets were formulated to contain 48% protein and 21% lipid using the same ingredient composition except for the added lipid source. Oils used for the experimental diets came from two different vegetable sources with different degrees of saturation, palm (P) and rapeseed (R). For each source, four different types of oil were used: native oil (N), re-esterified oil low in MAG (EL), re-esterified oil high in MAG (EH) and acid oil (A), all resulting in eight experimental diets (Table 1). Commercial fish oil was used for the control diet (FO). Native, acid and re-esterified oils were provided by SILO S.p.a. (Firenze, Italy). Both native palm and rapeseed oils were crude oils. In the process of re-esterification, the proportion fatty acid:glycerol was fixed to obtain a re-esterified oil with a high MAG content (EH oil) and a re-esterified oil with a low MAG content (EL oil). The free fatty acidity was determined following the ISO 660:1996 method. Glycerol was calculated according to the following stoichiometric formula: glycerol weight = fatty acid weight · free fatty acid acidity · glycerol molecular weight / fatty acid molecular weight. Once the proportion fatty acids:glycerol was established, both components were put in the reactor at 190–205 °C of temperature and 1–3 mm Hg of pressure for 4–6 h. Feeds were produced at the Skretting Feed Technology Plant (Aquaculture Research Center; Stavanger, Norway) as extruded pellets. Yttrium oxide (Y_2O_3) was added to the diets as an inert marker for the apparent digestibility of fatty acid measure. The ingredient formulation and proximate composition of the diets are shown in Table 1. Nutrient composition of experimental diets was determined by standard procedures (AOAC, 2005): moisture (934.01), ash (942.05), crude protein (968.06) and crude lipid (920.39). Gross energy of dried feed was determined using an adiabatic bomb calorimeter (IKA-Kalorimeter system C4000, Jankel-Kunkel, Staufen, Germany). Yttrium was analyzed according to Austreng et al. (2000).

2.2. Fish, experimental conditions and sampling

All the procedures were conducted in accordance with the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona (UAB) and following the European Union Guidelines for the ethical care and handling of animals under experimental conditions (2010/63/EU). The trial was carried out at the Skretting Aquaculture Research Center in Mozzecane, Italy. A total of 567 rainbow trout with a mean initial body weight of 412.7 ± 54 g were randomly distributed into 27 cylindro-conical tanks of 600 l of capacity (21 fish per tank) in an open freshwater system with a continuous water flow of 24 l min^{-1}. Water temperature (14.3 °C) and dissolved oxygen levels (7.15 ± 0.2 mg/l) were maintained constant throughout the experimental period. The tanks were subjected to a 24 h light photoperiod. Fish were fed the experimental diets for 21 days. Each diet was randomly assigned to three replicate tanks and was fed twice a day by automatic feeders, adjusted to provide the 2.5% of biomass daily. Uneaten feed was collected by filtering effluent water from each tank. Collectors were emptied after each meal and feed intake was recorded daily. At the end of the experimental period, all the fish from each tank were anaesthetized with clove oil (Phytosynthese, Za de Mozac-Volvic, France; 0.04 ml/l) and faecal samples were collected from the hindgut by manual stripping. After faecal stripping, fish were put into tanks supplied with freshwater to recover from anaesthesia. Faecal samples were pooled by tank and stored at −20 °C prior to analysis of yttrium oxide, fatty acid composition and gross energy.

2.3. Fatty acid composition

Fatty acid composition of oils, diets and faeces was determined by gas chromatography-flame ionization detector (GC-FID). For experimental oils, the fatty acid methyl esters (FAMEs) were previously obtained as described by Vilarrasa et al. (2014). For diets and faeces, FAMEs were obtained by direct methylation, according to Meier et al. (2006) and analyzed using an HP 5890A gas chromatograph. In both cases, fatty acid methyl esters were identified by comparison of their