Lipid digestion in turbot (Scophthalmus maximus). I: Lipid class and fatty acid composition of digesta from different segments of the digestive tract

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Abstract

The lipid content and lipid composition of digesta from the stomach, foregut, hindgut and rectum of juvenile turbot fed a commercial diet were determined in order to examine the process of lipid digestion in this species. The moisture content of the digesta increased along the digestive tract from 71.5% in the stomach to 89.6% in the rectum. The lipid content of the digesta increased initially from 15.7% of the dry weight in the stomach to 36.1% in the foregut but thereafter decreased through 23.2% in the hindgut to 9.1% in the rectum. The proportion of triacylglycerols (TAG) in the total lipid of the digesta decreased from 63% in the stomach to 17.4% in the rectum whereas that of free fatty acids (FFA) increased from 10% to 48.9%. The highest proportions of monoacylglycerols (MAG), diacylglycerols (DAG) and most phospholipids were observed in the lipid of the hindgut digesta. In addition, a fall in levels of neutral and phospholipid classes as digesta moved from hindgut to rectum signified absorption.

Analysis of the fatty acid composition of the lipid classes TAG, DAG and MAG suggest a polyunsaturated fatty acid specificity for hydrolysis may exist. Saturated, monounsaturated and polyunsaturated fatty acids (PUFA) accounted for 17.9%, 45.4% and 37.0%, respectively of the FFA present in the foregut whereas the corresponding values for the rectum were 32.6%, 51.9% and 16.3%. Overall, the results suggest a PUFA specificity for hydrolysis may exist alongside the positional non-specific lipolytic activity associated with the hindgut regions of the digestive tract of turbot and that PUFA, released by lipolysis are more effectively absorbed from the digesta than monounsaturated and saturated fatty acids.

Introduction

A considerable fraction of the energy intake of marine carnivorous fish is in the form of lipid containing high concentrations of (n-3) polyunsaturated fatty acids (PUFA). Consequently, lipase activity has been detected in the digestive tract of various marine fish species (Chesley 1934; Kitamikado and Tachino 1961; Brockerhoff 1966; Sastry 1974; Mukhopadhyay 1977; Olatunde and Ogunbiyi 1977; Borlongan 1990). Parallels have been drawn with the main mammalian lipolytic enzyme, pancreatic lipase, which cleaves the outer or α positions of triacylglycerols yielding a mixture of α,β diacylglycerols, β monoacylglycerols and free fatty acids that are subsequently absorbed in the intestinal wall of the jejunum (Gurr and Harwood 1991). Although the teleost pancreas is generally diffuse, fish digestive lipases were assumed to be similar to mammalian lipases (Brockerhoff 1966) and this was
demonstrated in several species (Brockerhoff and Hoyle 1965; Brockerhoff 1966; Tocher and Sargent 1984). It followed that lipid digestion and absorption were shown to take place primarily in the anterior intestine (Mukhopadhyay 1977; Fange and Grove 1979; Buddington and Doroshov 1986; Das et al. 1987; Borlongan 1990). However, the PUFA of marine lipids are not readily hydrolysed, irrespective of position, by mammalian 1,3 specific pancreatic lipase \textit{in vitro} (Bottino et al. 1967; Chen et al. 1990) suggesting that a lipase other than pancreatic lipase is the major lipolytic enzyme in fish (Patton et al. 1975). In fact, some teleosts can secrete intestinal mucosal lipase (Sastry 1974; Kapoor et al. 1975) that can be active throughout the digestive tract (Munilla-Moran and Stark 1990).

Fish lipases can vary in their lipolytic activity showing a combination of positional and fatty acid specificity. Sharks produce both a non-specific bile-activated lipase preferentially lysing PUFA and another exhibiting the classical specificity for primary esters (Patton and Nevenzel 1974). Pancreatic rainbow trout tissue demonstrated a preferred hydrolysis of PUFA from triacylglycerols (Leger and Bauchard 1972) while pyloric caeca extracts of anchovy and salmon hydrolysed 2-monoacylglycerols more effectively than mammalian lipase (Patton et al. 1975). Studies on cod (Lie and Lambertsen 1985; Lie et al. 1987; Gjellesvik et al. 1992) reported a bile salt-dependent pancreatic lipase (BSDL) preferentially hydrolysing PUFA regardless of position in the triacylglycerol molecule while saturated fatty acids were more resistant to hydrolysis. Thus, the lipolytic breakdown and absorption of dietary lipids in fish, in general, may not be strictly analogous to that in mammals.

Turbot, \textit{Scophthalmus maximus}, is a commercially valuable teleost where the understanding of digestion and absorption of lipids is still inadequately known. Furthering the knowledge of these processes would clearly benefit the formulation of more effective artificial feeds. The present work examined the lipid class and fatty acid compositions of digesta from the stomach, foregut, hindgut and rectum of the alimentary canal in turbot with the aim of providing information on the process of lipid digestion in turbot.

**Materials and methods**

Juvenile turbot (50–60 g), purchased from Golden Sea Produce, Hunterston, U.K., were maintained at 14.5 ± 1.5°C in a 200 l rectangular PVC tank with aeration and an inflow of 3 l/min of recirculated filtered seawater. The fish were fed to satiation on 5 mm commercial trout feed\(^1\) every other day. In each of three separate samplings, five fish, after consuming 6 pellets/fish, were removed to a smaller 80 l holding tank at 18.5 ± 0.3°C with aeration and an inflow of 2 l/min. In previous trials the digestive tract from stomach to rectum was observed full of digesta after 14 h under the prevailing experimental conditions. As a result, three of the five fish were killed after this time period, their digestive tracts dissected out and the digesta from the stomach, foregut, hindgut and rectum (Fig. 1) extruded separately. The replicate samples of digesta from each segment were pooled and kept frozen until analyzed for moisture (%), total lipid (%DW), lipid class (% of total lipid and mg/g DW digesta) and fatty acid composition (% wt/lipid class).

Moisture determinations were carried out on ca. 200 mg of digesta by standard methods (Horowitz 1980). Total lipid in digesta was determined gravimetrically (± 0.001 mg) after extraction (Folch et al. 1957) with chloroform:methanol (2:1, v/v) and evaporation to dryness under a stream of \textit{N}_2. Prior to analysis, total lipid extracted from digesta (100–400 mg) was redissolved in chloroform: methanol (2:1, v/v) to a concentration of 10 mg ml\(^{-1}\) and stored at -20°C.

For the analysis of lipid class composition, aliquots (15 µg) of the lipid fraction together with the appropriate standards were loaded, using a 10 µl microsyringe, as 2 mm streaks onto 10 × 10 cm high performance thin layer chromatography (HPTLC) plates precoated with silica gel G60 without fluorescent indicator (E. Merk, Darmstadt, F.R.G.). Double development of the chromatograms was performed at room temperature in saturated standard chambers for 10 × 10 cm HPTLC plates using \(^1\)Fatty acid analysis of dietary lipid fraction suggests only polyunsaturated oils such as herring oil were used for its manufacture.