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ORIGINAL ARTICLE

Dietary Lipid and Carbohydrate Interactions: Implications on Lipid and Glucose Absorption, Transport in Gilthead Sea Bream (*Sparus aurata*) Juveniles

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Abstract A digestibility trial was performed with gilthead sea bream juveniles (IBW = 72 g) fed four diets differing in lipid source (fish oil, FO; or a blend of vegetable oil, VO) and starch content (0 %, CH−; or 20 %, CH+) to evaluate the potential interactive effects between carbohydrates and VO on the processes involved in digestion, absorption and transport of lipids and glucose. In fish fed VO diets a decrease in lipid digestibility and in cholesterol (C), High Density Lipoprotein(HDL)-C and Low Density Lipoprotein (LDL)-C (only in CH+ group) were recorded. Contrarily, dietary starch induced postprandial hyperglycemia and time related alterations on serum triacylglycerol (TAG), phospholipid (PL) and C concentrations. Fish fed a CH+ diet presented lower serum TAG than CH− group at 6 h post-feeding, and the reverse was observed at 12 h post-feeding for TAG and PL. Lower serum C and PL at 6 h post-feeding were recorded only in VOCH+ group. No differences between groups were observed in hepatic and intestinal transcript levels of proteins involved in lipid transport and hydrolysis (*FABP, DGAT, GPAT, MTP, LPL, LCAT*). Lower transcript levels of proteins related to lipid transport (*ApoB, ApoA1, FABP2*) were observed in the intestine of fish fed the CH+ diet, but remained unchanged in the liver. Overall, transcriptional mechanisms involved in lipid transport and absorption were not linked to changes in lipid serum and digestibility. Dietary starch affected lipid absorption and transport, probably due to a delay in lipid absorption. This study suggests that a combination of dietary VO and starch may negatively affect cholesterol absorption and transport.

Keywords Alternative ingredients · Digestibility · Gene expression · Nutrient absorption and transport · Serum metabolites

Abbreviations

ADC	Apparent digestibility coefficients
Apo	Apolipoproteins
CH	Carbohydrates
C	Cholesterol
C18 PUFA	Polyunsaturated fatty acids with 18 carbons
DGAT	Diacylglycerol acyltransferase
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FABP	Fatty acid binding protein
FM	Fish meal
FO	Fish oil
GLU	Glucose
GPAT	Glycerol-3-phosphate acyltransferase
G3P	Glycerol-3-phosphate
HDL	High-density lipoproteins
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low-density lipoproteins
LC-PUFA	Long-chain polyunsaturated fatty acids
LPL	Lipoprotein lipase
MTP	Microsomal triglyceride transfer protein
MUFA	Monounsaturated fatty acids
NEFA	Non-esterified fatty acids

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PL	Phospholipid
SFA	Saturated fatty acids
TAG	Triacylglycerol
VLDL	Very low density lipoprotein
VO	Vegetable oil

Introduction

The fish gastrointestinal tract is involved in nutrient assimilation, digestion and absorption, while liver is the central organ involved in nutrient metabolism. Apart from other functions, both organs constitute critical interfaces in the control of glucose and lipid homeostasis [1, 2]. Fish in general digest dietary lipids efficiently [3, 4]. Despite the poor utilization of dietary carbohydrates by carnivorous fish, high carbohydrate (gelatinized starch) digestibility has been reported in gilthead sea bream (*Sparus aurata*) and other species [5–8]. The general mechanisms involved in digestion and absorption of dietary lipids and carbohydrates have been verified in all fish species investigated to date, namely the enzymatic machinery for lipid and carbohydrate hydrolysis and a system of specialized protein transporters, such as fatty acid binding proteins and glucose transporters [1, 3, 4, 9]. Most of the processes involved in the digestion and absorption of lipids and carbohydrates occur predominantly in the proximal and middle intestine [1, 4, 10, 11]. As in mammals, lipid digestion products mostly accumulate in the enterocytes as lipid droplets. These need to be resynthesized into lipoprotein particles, as triacylglycerol (TAG)-rich lipoproteins (chylomicrons), to be exported through the vascular system to the liver which is the interface between the exogenous pathway (dietary lipids) and the endogenous pathway. The endogenous pathway mediates the transport of lipids from the liver, where both dietary lipids and *de novo* synthesized lipids are incorporated into TAG-rich lipoproteins (VLDL), to the sites of conversion, storage or energy utilization [12, 13]. Lipoproteins are lipid complexes with a neutral lipid core consisting of TAG and cholesteryl esters surrounded by a surface monolayer of phospholipid (PL)s, small amounts of unesterified cholesterol and specialized proteins - apolipoproteins (Apo) [2]. The association of lipids and protein during lipoprotein synthesis is a complex multistep process that involves several proteins, such as *ApoB*, *ApoA1*, microsomal transfer protein, *MTP* (for details see Gu *et al*. [14] and Mansbach and Gorelick [15]). These proteins were shown to be highly expressed both in the liver and in the intestine of different fish species [5, 8, 14, 16, 17]. Once in blood circulation lipoproteins, especially TAG-rich VLDL and chylomicrons, suffer intra-vascular transformations that lead to modifications in the lipoprotein composition (TAG, PL, cholesterol, apolipoproteins) resulting in the formation of low-density (LDL) and high-density (HDL) lipoproteins by the action of lipoprotein lipase (*LPL*) and lecithin-cholesterol acyltransferase (*LCAT*) [12].

It has been demonstrated that dietary carbohydrate and lipid source induce changes in nutrient digestibility [7, 18–20] and in serum metabolites and lipoprotein profiles [5, 20–26]. Most of these changes are thought to be linked to intracellular events involved in lipid and glucose absorption and transport (as described above) and that were shown in different fish studies to be under nutritional control, at least at the transcriptional level [8, 17, 27–29]. For instance, carbohydrate intake induced an increase in midgut *ApoA1* and of hindgut *ApoA1* and *ApoB* transcript levels in rainbow trout (*Oncorhynchus mykiss*) fed FO-based diets [8]. Furthermore, carbohydrate intake increased intestinal transcript levels of *MTP, ApoA1* and *ApoA4*, along with higher serum cholesterol concentrations in rainbow trout fed VO-based diets [17]. In Atlantic salmon (*Salmo salar*), replacement of fish oil (FO) by rapeseed oil (RO) promoted a decrease in the hepatic expression of *MTP2* and *ApoA1* genes [28], while replacement of FO with RO or linseed oil (LO) induced an up-regulation of hepatic transcript levels of *ApoB* [29]. Similarly, up-regulation of hepatic transcript levels of *ApoB* and *ApoA1* was reported in European sea bass (*Dicentrarchus labrax*) fed plant-based diets [27].

Fish meal (FM) and FO have traditionally been used as reliable sources of protein and lipids in carnivorous fish feeds. However, the limited availability of fisheries by-products together with the escalating cost and demand of these commodities made it critical to develop aquafeeds rich in more sustainable ingredients such as vegetable protein and oil sources. Vegetable feedstuffs are usually rich in carbohydrates and, contrary to FO, do not have n-3 long-chain polyunsaturated fatty acids (LC-PUFA) [30, 31]. Although a number of studies have demonstrated the potential of vegetable feedstuffs as alternative ingredients in aquafeeds there are still problems related to their use for carnivorous fish, particularly due to limitations in their carbohydrate metabolism [32–34] and LC-PUFA biosynthesis [31, 35]. As dietary inclusion of VO and carbohydrate are particularly challenging in diets for carnivorous fish, such as gilthead sea bream, more knowledge is needed on the potential implications of dietary carbohydrates and VO, and of the potential interaction between them, on the processes involved in lipid and glucose digestion, absorption and transport. Thus, this study aimed to evaluate the effects of dietary carbohydrate content and lipid source on digestibility, serum metabolites, hepatic and intestinal expression of key proteins involved in lipid and glucose absorption and transport in juveniles of an economically important carnivorous fish species of aquaculture in Europe, gilthead sea bream.

Materials and Methods

Experimental Diets

Four diets differing in carbohydrate content (0 and 20 % gelatinized maize starch, CH− and CH+ , respectively) and lipid source (fish oil or a vegetable oil blend, FO and VO, respectively) were formulated (Table 1). The diets were isolipidic and the dietary carbohydrate content was increased at the expense of dietary protein, which was always above the 45–46 % protein requirement of this species [36]. Fish meal was added as a major dietary protein source to isolate the impacts of dietary VO and to avoid the interference of dietary plant protein on lipid metabolism. The major lipid source of FO diets was cod liver oil. In VO diets, 100 % of the cod liver oil was replaced by a VO blend composed of 20 % rapeseed, 50 % linseed and 30 % palm oils. The diets presented small differences in the proportions of total saturated fatty acids (SFA), which were slightly higher in VO diets, and of monounsaturated fatty acids (MUFA) which were higher in FO diets (Table 2). Within MUFA, levels of oleic acid (18:1n-9) were higher in the VO diets while the opposite occurred for palmitoleic acid (16:1n-7), eicosenoic acid (20:1n-9) and erucic acid (22:1n-9). Linoleic acid (18:2n-6) was considerably higher in VO diets than in FO diets. Compared to the FO diets, the VO diets were rich in linolenic acid (18:3n-3) and poor in eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid DHA (22:6n-3). Dietary cholesterol content was higher in the diets without starch supplementation, particularly in the FOCH- diet.

Cr_2O_3 was incorporated in the diets as an indigestible marker for digestibility measurement of dietary components.

All ingredients were finely ground, well mixed, and dry extruded in a laboratory pellet mill (California Pellet Mill, Crawfordsville, IN, USA), through a 3-mm die. The pellets were air dried for 24 h and stored in a refrigerator (4 °C) until used.

Digestibility Trial

The digestibility trial was performed at the Marine Zoology Station, Porto University, in a thermo-regulated recirculation water system equipped with a battery of 12 fiberglass tanks of 60 L capacity designed according to Cho *et al.* [37] and with a feces settling column connected to the outlet of each tank.

The trial was performed with gilthead sea bream juveniles with a mean initial body mass of 72 g. Before the trial the fish were acclimatized for 15 days to the tanks and rearing conditions and were fed a commercial diet. Thereafter, 22 fish were randomly distributed to each tank and each diet was randomly assigned to triplicate groups of these fish. During the trial, water temperature averaged 24.0 ± 0.5 °C, salinity averaged 34.7 ± 0.8 g L^{-1} and dissolved oxygen was kept near saturation. Fish were hand fed to satiation twice a day. The first 15 days of the trial were

Table 1 Ingredient and chemical composition of the experimental diets

Lipid source	Experimental diets			
Carbohydrates	FO		VO	
	CH−	CH+	CH−	CH+
Ingredients (% dry weight)				
Fish meal[a]	86.8	64.5	86.8	64.5
Gelatinized maize starch[b]	0	20	0	20
Cod liver oil[c]	9.2	11.5	0	0
Vegetable oil blend[d]	0	0	9.2	11.5
Vitamins[e]	1.5	1.5	1.5	1.5
Minerals[f]	1.0	1.0	1.0	1.0
Binder[g]	1.0	1.0	1.0	1.0
Cr_20_3	0.5	0.5	0.5	0.5
Proximate analyses (% dry matter)				
Dry matter (DM)	84.1	84.7	83.9	84.8
Crude protein (CP)	66.2	51.3	65.6	50.2
Crude fat (CF)	17.9	17.9	17.2	17.8
Starch	–	17.5	–	17.3
Gross energy (MJ kg^{-1})	23.0	22.6	23.2	22.7
Ash	14.6	11.8	15.1	11.9
Cholesterol	0.7	0.4	0.5	0.4

Fish oil (FO), blend of vegetable oils (VO); carbohydrate content: 0 % (CH−) or 20 % (CH+) gelatinized maize starch

[a] Steam dried LT fish meal, (Superprime). Inproquisa, Madrid, Spain Pesquera Diamante, Perú (CP: 74.6 % DM; CL: 10.1 % DM)

[b] C-Gel Instant-12018, Cerestar, Mechelen, Belgium

[c] Labchem, Laborspirit Lda, Lisboa, Portugal

[d] 30 % palm oil (Colmi, Malasia), 50 % linseed oil (Sociedade Portuense de Drogas, S.A.,Portugal) and 20 % rapeseed oil (Huilerie Emile Noël S.A.S., France)

[e] Vitamins (mg kg^{-1} diet): retinol acetate, 18,000 (IU kg^{-1} diet); cholecalciferol, 2000 (IU kg^{-1} diet); alpha tocopherol acetate, 35; sodium menadione bisulphate, 10; thiamin-HCl, 15; riboflavin, 25; calcium pantothenate, 50; nicotinic acid, 200; pyridoxine HCl, 5; folic acid 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbic acid, 50; inositol, 400. Premix, Viana do Castelo, Portugal

[f] Minerals (mg kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dibasic calcium phosphate, 5.93 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.40 (g kg^{-1} diet). Premix, Viana do Castelo, Portugal

[g] Aquacube (Guar gum, polymethyl carbamide, Manioc starch blend, hydrate calcium sulphate). Agil, England

Table 2 Fatty acid composition (expressed as mol%) of the experimental diets

Lipid source	Experimental diets			
Carbohydrates	FO		VO	
	CH−	CH+	CH−	CH+
14:00	7.3	7.3	3.2	2.6
15:00	0.8	0.7	0.5	0.4
16:00	21.7	19.8	24.5	23.9
17:00	0.6	0.5	0.5	0.4
18:00	4.2	3.6	5.1	4.7
20:00	0.2	0.1	0.3	0.2
∑SFA	34.7	32.1	34.1	32.4
16:1 n-7	8.2	8.4	2.7	2.0
18:1 n-9	19.1	20.5	26.9	28.8
20:1 n-9	4.7	5.3	0.8	0.7
22:1 n-9	3.3	3.7	0.4	0.4
∑MUFA	35.2	37.9	30.8	31.8
18:2 n-6	2.3	2.8	9.7	11.1
18:3 n-6	0.1	0.2	0.0	0.1
20:2 n-6	0.2	0.3	0.1	0.1
20:4 n-6	1.1	0.9	0.9	0.6
∑n-6 PUFA	4.1	4.3	10.8	12.0
18:3 n-3	1.2	2.0	17.0	20.1
18:4 n-3	2.0	2.2	0.5	0.4
20:4 n-3	0.5	0.6	0.2	0.1
20:5 n-3	7.5	7.2	3.2	2.3
21:5 n-3	0.3	0.3	0.1	0.1
22:5 n-3	1.1	1.0	0.6	0.4
22:6 n-3	10.5	9.5	6.0	4.4
∑n-3 PUFA	23.3	23.0	27.6	28.0
Sat/PUFA	1.2	1.1	0.9	0.8
n3/n6	5.7	5.3	2.6	2.3

Fish oil (FO), blend of vegetable oils (VO); carbohydrate content: 0 % (CH−) or 20 % (CH+) gelatinized maize starch

SFA saturated fatty acids; *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n-3 LC-PUFA* n-3 long chain polyunsaturated fatty acids

used for fish to adapt to the diets; then, feces were collected once a day before the morning meal during the following 17 days. Immediately after collection feces from each tank were centrifuged, pooled for the whole period, and stored at −20 °C until analysis.

Tissues and Blood Sampling

After 36 days of being fed with the experimental diets, blood samples were collected at 2, 4, 6, 8, 12 and 24 h after feeding for analysis of serum metabolites. Blood was collected from the caudal vein by puncture with a heparinized syringe and immediately centrifuged at 1800*g* for 12 min, and serum aliquots were stored at 4 °C until analysis. In order to minimize stress due to sampling, at each sampling point blood was collected from 9 fish from one tank and that tank was only sampled again after at least 6 h.

The remaining fish continued to be fed for two more days and then two fish from each tank were randomly sampled 6 h after the last meal to collect liver and intestine, just after the last pyloric caecum, for gene expression analysis. Immediately after collection, tissue samples were frozen in liquid nitrogen and then stored at −80 °C until analysis.

Analytical Methods

Details on diet and feces analysis are given in Castro *et al.* [5]. Apparent digestibility coefficients (ADC) were calculated according to the formula:

$$\mathrm{ADC}\ (\%) = 100 - \left[100 \times (\mathrm{Cr_2O_{7diet}} / \mathrm{Cr_2O_{7feces}}) \times (\mathrm{N_{faeces}}/\mathrm{N_{diet}})\right]$$

where N is nutrient (protein, lipid), starch or dry matter.

Serum Metabolites

Serum total cholesterol (C), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), TAG, PL, non-esterified fatty acids (NEFA) and glucose) were performed on a Clinical Laboratory certified NP EN ISO 9001-2000 by Bureau Veritas, following standard clinical methods and using an auto-analyzer (Architect ci8200; Abbot Diagnostics, Canada).

Gene Expression

Analyses of mRNA levels were performed on liver and intestine samples (2 fish/tank). Tissues for RNA analyses were homogenized in 2 mL tubes containing Trizol reagent (Invitrogen, Carlsbad, CA, USA) using rapid vibration (liver: 2 × 10 s, with an interval of 10 s, at 5000 rpm; intestine: 3 × 10 s, with an interval of 10 s, at 6500 rpm) in a Precellys®24 (Bertin Technologies, Montigny-le- Bretonneux, France). The extraction of total RNA was then performed according to manufacturer recommendations. RNA quality and quantity were assessed by gel electrophoresis and spectrophotometry (NanoDrop ND-1000, Nanodrop Labtech, Palaiseau, France). Complementary DNA (cDNA) synthesis was performed with 1 µg of the resulting total RNA using SuperScript III RNaseH-Reverse Transcriptase kit (Invitrogen) and random primers (Promega, Charbonnières, France). Gene expression levels were determined by real-time quantitative PCR (q-PCR) using LightCycler® 480 II apparatus (Roche Diagnostics, Neuilly sur Seine, France). Analyses were performed using 2 µL of the diluted cDNA (1:76) mixed with 0.24 µL of each primer (10 µM), 3 µL LightCycler® 480 SYBR Green I Master (Roche

Table 3 Sequences of the primer pairs used for real-time quantitative PCR determination of the transcript level of several gilthead sea bream genes

Gene	5′–3′ forward primer	5′–3 reverse primer	EL	EI	Acession number
ApoA1	GAATACAAGGAGCAGATGAAGCAGATG	TGGTGACGGAGGCAGCGATG	1.97	1.96	Varó *et al.* [39]
ApoB	ACTCTGAAGGCTGTTGTCGA	GCTGCAGGAAACCAGAAACA	1.90	1.97	CV133385[a]
DGAT	GAGCCAATCACTGTGCCAAA	TGAGGAGGGTCTGAAGATGC	1.93	1.97	AM957740[a]
EF1α	CATGCTGGAGACCAGTGAAA	CGGGTACAGTTCCAATACCG	1.95	1.92	Enes *et al.* [40]
FABP	AAATGGTTGAGGCTTTCTGTGCTAC	ATCGCTACTGTCGGCTTGGTG	1.84	1.87	Varó *et al.* [39]
FABP2	CGAGCACATTCCGCACCAAAG	CCCACGCACCCGAGACTTC	[b]	1.99	Pérez-Sánchez *et al.* [41]
GPAT	GATCCAGTACGGAGTTCTCTACG	AAAGGGGCTCGGGAAACT	2.00	1.98	Mininni *et al.* [42]
LCAT	CCAACATCAAGATCCGCGAG	ACACCACACCTCTACACCAG	1.94	1.96	AM950576[a]
LPL	CGTTGCCAAGTTTGTGACCTG	AGGGTGTTCTGGTTGTCTGC	1.79	1.85	Pérez-Sánchez *et al.* [43]
MTP	AGCCATCGACATCTCTGGAG	TTGTCCATCTGCATGCACAC	2.00	1.80	CB184347[a]
PLA2	CCAGACCATCTTCACCATCC	CACCCAATCCACAGGAGTTC	1.90	2.00	AM972037[a]

Primer efficiency in liver (EL) and intestine (EI), respectively

[a] Sigenae accession no. *ApoA1* apolipoprotein A-1, *EF1α* elongation factor 1α, *FABP* fatty acid-binding protein, *GPAT* glycerol-3-phosphate acyltransferase, *LCAT* lecithin-cholesterol acyltransferase, *LPL* lipoprotein lipase, *MTP* microsomal triglyceride transfer protein, *PLA2* phospholipase A2;- no expression

[b] Not assessed

Diagnostics GmbH, Mannheim, Germany) and 0.52 μL DNase/RNase/Protease-free water (5 prime GmbH, Hamburg, Germany) in a total volume of 6 μL. Primers were either obtained in the literature or designed from gilthead sea beam expressed sequence tag (EST) sequences available on the SIGENAE database (http://www.sigenae.org) using Primer3 software [38] (Table 3). For gene targets that had not been previously validated, primers were tested on a pool of cDNA and amplified products were systematically sequenced.

Thermal cycling was initiated with incubation at 95 °C for 10 min for hot-start iTaq™DNA polymerase activation. 45 steps of PCR were then performed, each one consisting of heating at 95 °C for 15 s for denaturing, at 60 °C for 10 s for annealing, and at 72 °C for 15 s for extension. Following the PCR cycle, melting curves were systematically monitored (55 °C temperature gradient at 0.5 °C 5 s^{-1} from 55 to 94 °C) to ensure that only one fragment was amplified. Each PCR run included duplicates of reverse transcription for each sample and negative controls (reverse transcriptase-free samples, RNA-free samples). The PCR run for reference gene included quadruplicates for each sample (duplicates of reverse transcription and PCR amplification, respectively) and negative controls. Quantification of the target gene transcripts in the liver was done using the *elongation factor 1α (EF1α)* gene expression as reference, as previously used in gilthead sea bream by Enes *et al.* [40], and that was stably expressed in the present study (data not shown). Relative quantification of the target gene transcript with the *EF1α* reference gene transcript was performed using the mathematical model described by Pfaffl [44]. The relative expression ratio (R) of a target gene was calculated on the basis of real-time PCR efficiency (E) and the CT deviation (ΔCT) of the unknown sample compared with a control sample and expressed in comparison with the *EF1α* reference gene:

$$\begin{aligned} R = \Big[& (\text{Etarget gene})\Delta\text{CT target gene (mean control} \\ & - \text{mean sample})\,]/[\,(\text{Eef1}\alpha)\Delta\text{CT EF1}\alpha \\ & \times (\text{mean control} - \text{mean sample})\Big]. \end{aligned}$$

Efficiency of q-PCR was measured by the slope of a standard curve using serial dilutions of cDNA.

Statistical Analysis

Data are presented as means ± standard deviations. Data were checked for normal distribution and homogeneity of variances and when appropriate normalized. The apparent digestibility coefficients and gene expression data were analyzed by two-way ANOVA, with lipid source and carbohydrate level as fixed factors. The effects of diet and sampling time on serum metabolites level were analyzed by three-way ANOVA, with lipid source, carbohydrate level and sampling time as fixed factors. In the case of significant interactions one-way ANOVA was performed for each fixed factor. Significant differences between sampling times were evaluated by the Tukey multiple range test. The significance level of 0.05 was used for rejection of the null hypothesis. All statistical analyses were done using the SPSS 22.0 software package (IBM Corp., New York, NY, USA) for Windows.

Table 4 Apparent digestibility coefficients (%) of the experimental diets

Lipid source (LS)	Experimental diets				*p* value*		
Carbohydrates (CH)	FO		VO				
	CH−	CH+	CH−	CH+	CH	LS	CH × LS
Dry matter	73.5 ± 6.0	81.0 ± 3.0	69.1 ± 7.2	74.1 ± 8.2	0.126	0.173	0.726
Protein	85.8 ± 3.6	85.6 ± 3.4	82.1 ± 7.5	81.2 ± 7.8	0.890	0.308	0.947
Lipid	96.7 ± 1.1	97.4 ± 0.2	94.7 ± 1.3	94.7 ± 1.1	0.512	0.003	0.465
Starch	–	92.1 ± 0.9	–	90.0 ± 1.5	–	0.109[a]	–

Fish oil (FO), blend of vegetable oils (VO); carbohydrate content: 0 % (CH−) or 20 % (CH+) gelatinized maize starch

Values are presented as means ($n = 3$) and standard deviation (SD) and were analyzed by two-way ANOVA

[a] p value assessed by Student's t-test: effect of lipid source $p = 0.109$. values not showing an asterisk are non-statistically significant ($p > 0.05$)

* Differences were considered statistically significant at: $p < 0.05$

Results

Mortality was lower than 6 % and it was negatively affected by dietary VO administration in fish fed carbohydrate-rich diets (data not shown).

The ADC of nutrients was high, irrespective of dietary treatments (Table 4). The ADC of dry matter, protein and starch were not affected by diet composition, but the ADC of lipids was lower in the VO diets.

Postprandial serum glucose (GLU), TAG, C, PL, NEFA, LDL-C and HDL-C levels at 2, 4, 6, 8, 12 and 24 h after feeding are presented in Fig. 1 and the summary of statistical analysis is presented in Table 5.

Postprandial serum GLU levels in CH+ groups increased to a peak at 4 h post-feeding and remained higher until 8 h post-feeding; thereafter GLU decreased to basal values which was attained 12 h post-feeding. In CH− group GLU levels remained relatively constant, though a small peak was observed at 8 h post-feeding. While during the first 8 h after feeding CH+ groups exhibited higher serum GLU concentrations than CH− groups, at 12 h and 24 h post-feeding no differences or the reverse pattern was observed, respectively (CH × time interaction). In CH− groups, increased levels of GLU were found in fish fed VO based diet (CH × LS interaction).

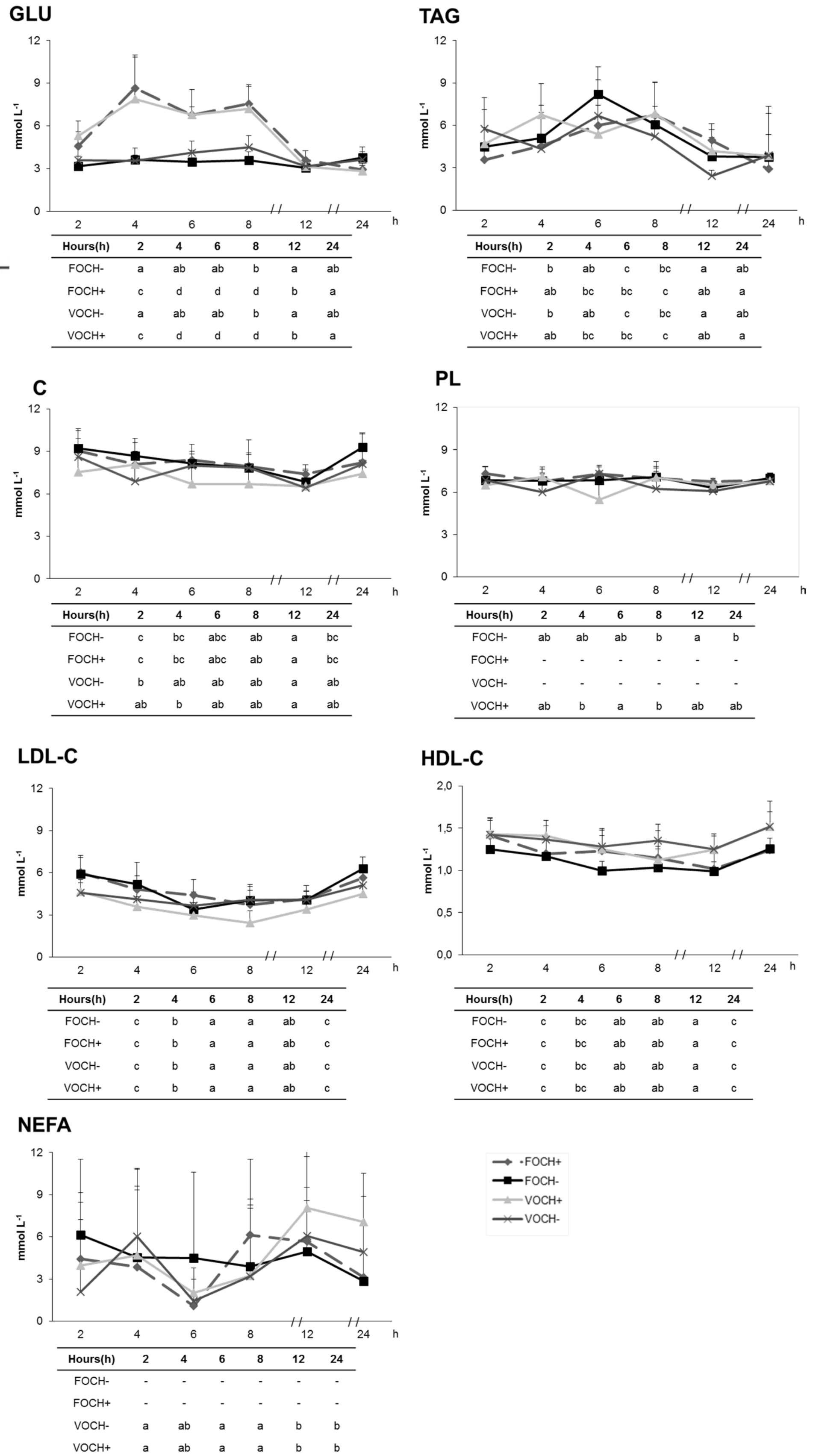
Serum TAG in CH+ groups attained a peak at 8 h post-feeding and decreased to the basal level at 24 h post-feeding. In CH− groups serum TAG peaked at 6 h post-feeding and decreased to basal levels 12 h post-feeding. Dietary carbohydrate affected TAG concentrations only at 6 h and 12 h post-feeding (CH × time interaction). Increased serum TAG was observed in CH− groups at 6 h after feeding, while the reverse was observed at 12 h post-feeding. TAG concentration was not affected by dietary lipid source.

All diets followed a similar postprandial C profile. Accordingly, serum C decreased to basal values 12 h after feeding, and 24 h post-feeding increased to values similar to those observed 2 h after feeding. The VOCH+ group followed a similar C pattern, except that a peak of C was observed at 4 h post-feeding. Serum C concentration was lower in VO groups than FO groups, except at 8 and 24 h post-feeding. Serum C was also affected by dietary carbohydrate but only in VO groups. Thus, 4 h post-feeding higher C concentration was observed in VOCH+ group than in VOCH− group while the opposite was observed 6 h post-feeding (CH × LS × time interaction).

Postprandial differences in PL concentration were observed in FOCH− and VOCH+ groups. In VOCH+ group, two peaks of PL concentration were observed, the first one at 4 h and the second one at 8 h post-feeding, while in FOCH− group PL peaks were also observed at 8 h and at 24 h post-feeding. Serum PL concentration was affected by dietary carbohydrate only at 6 and 12 h post-feeding. Lower PL levels were observed in VOCH+ group 6 h post-feeding, while 12 h post-feeding higher PL was found in CH+ groups than in CH− groups (CH × LS × time interaction). Dietary VO promoted an effect on serum PL concentration only at 6 h post-feeding (CH × LS × time interaction). Accordingly, in the CH+ group, lower serum PL concentrations were observed, while the reverse was observed in the CH− group.

Postprandial differences in NEFA were only observed in VO groups, with NEFA levels being lower at 2, 6 and 8 h post-feeding than at 12 and 24 h post-feeding. Dietary carbohydrate did not affect serum NEFA concentration. However, NEFA concentration was lower at 2 h and higher at 24 h post-feeding in VO than in FO groups (LS × time interaction).

HDL-C and LDL-C exhibited similar postprandial profiles with the higher values being observed at 2 h and at 24 h after feeding. Within VO groups, carbohydrate intake induced lower serum LDL-C. In fish fed CH+ diets, VO



GLU

Hours(h)	2	4	6	8	12	24
FOCH-	a	ab	ab	b	a	ab
FOCH+	c	d	d	d	b	a
VOCH-	a	ab	ab	b	a	ab
VOCH+	c	d	d	d	b	a

TAG

Hours(h)	2	4	6	8	12	24
FOCH-	b	ab	c	bc	a	ab
FOCH+	ab	bc	bc	c	ab	a
VOCH-	b	ab	c	bc	a	ab
VOCH+	ab	bc	bc	c	ab	a

C

Hours(h)	2	4	6	8	12	24
FOCH-	c	bc	abc	ab	a	bc
FOCH+	c	bc	abc	ab	a	bc
VOCH-	b	ab	ab	ab	a	ab
VOCH+	ab	b	ab	ab	a	ab

PL

Hours(h)	2	4	6	8	12	24
FOCH-	ab	ab	ab	b	a	b
FOCH+	-	-	-	-	-	-
VOCH-	-	-	-	-	-	-
VOCH+	ab	b	a	b	ab	ab

LDL-C

Hours(h)	2	4	6	8	12	24
FOCH-	c	b	a	a	ab	c
FOCH+	c	b	a	a	ab	c
VOCH-	c	b	a	a	ab	c
VOCH+	c	b	a	a	ab	c

HDL-C

Hours(h)	2	4	6	8	12	24
FOCH-	c	bc	ab	ab	a	c
FOCH+	c	bc	ab	ab	a	c
VOCH-	c	bc	ab	ab	a	c
VOCH+	c	bc	ab	ab	a	c

NEFA

Hours(h)	2	4	6	8	12	24
FOCH-	-	-	-	-	-	-
FOCH+	-	-	-	-	-	-
VOCH-	a	ab	a	a	b	b
VOCH+	a	ab	a	a	b	b

Fig. 1 Postprandial serum glucose (GLU), triacylglycerol (TAG), total cholesterol (C), phospholipid (PL), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and non-esterified fatty acids (NEFA) concentrations in gilthead sea bream juveniles after feeding the —◆— FOCH+ —■— FOCH- —▲— VOCH+ —×— diets. Values are mean ± SD ($n = 9$). Results of statistical differences in postprandial serum metabolite profiles related to the effect of diet (lipid source and carbohydrate content), time point and interaction among the factors analysed by three-way analysis of variance-ANOVA ($P < 0.05$) are given in the "Results" section and Table 5. In the figure, within each diet, significant differences (three-way ANOVA followed by one-way ANOVA, in case of significant interaction, and Tukey's Post Hoc, $p < 0.05$) among sampling times are indicated by *different lowercase letters*

Table 5 Summary of the statistical analyses (3-way ANOVA) of postprandial serum glucose (GLU), triacylglycerol (TAG), total cholesterol (C), phospholipid (PL), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and non-esterified fatty acids (NEFA) in gilthead sea bream fed the experimental diets

	CH	L	Time	CH × L	CH × time	L × time	CH × L × time
p-value*							
GLU	<0.001	0.325	<0.001	0.007	<0.001	0.123	0.564
TAG	0.852	0.970	<0.001	0.066	0.003	0.081	0.723
PL	0.444	0.005	0.669	0.887	0.065	0.790	0.004
C	0.050	<0.001	<0.001	0.331	0.169	0.962	0.039
HDL-C	0.335	<0.001	<0.001	0.018	0.577	0.454	0.254
LDL-C	0.008	<0.001	<0.001	0.015	0.117	0.193	0.251
NEFA	0.534	0.982	0.028	0.153	0.304	<0.001	0.382

* *p*-value assessed by 3-way ANOVA. Differences were considered statistically significant at: $p < 0.05$

Table 6 Relative mRNA abundance of selected proteins involved in lipid digestion (*PLA2*), lipid transport (*FABP*), TAG and PL resynthesis (*DGAT, GPAT*), lipoprotein assembly (*ApoB, ApoA1, MTP*) and lipolysis (*LCAT, LPL*) in the liver of gilthead sea bream fed the experimental diets

Lipid source (LS)	Experimental diets				*p*-value*		
Carbohydrates (CH)	FO		VO				
	CH−	CH+	CH−	CH+	CH	LS	CH × LS
l-*ApoA1*	1.10 ± 0.42	1.38 ± 0.61	1.13 ± 0.31	1.30 ± 0.33	0.222	0.890	0.747
l-*ApoB*	1.08 ± 0.45	1.07 ± 0.29	1.18 ± 0.33	1.00 ± 0.15	0.652	0.715	0.513
l-*DGAT*	1.20 ± 0.95	1.42 ± 0.79	1.45 ± 0.47	1.30 ± 0.53	0.910	0.821	0.535
l-*FABP*	1.07 ± 0.50	0.83 ± 0.28	1.28 ± 0.44	0.90 ± 0.31	0.068	0.387	0.644
l-*GPAT*	1.13 ± 0.55	1.18 ± 0.47	1.05 ± 0.45	1.38 ± 1.57	0.443	0.321	0.206
l-*LCAT*	1.08 ± 0.46	1.45 ± 0.50	1.73 ± 0.47	1.13 ± 0.45	0.550	0.395	0.020
l-*LPL*	1.08 ± 0.46	1.38 ± 1.04	1.00 ± 0.18	0.97 ± 0.60	0.881	0.434	0.488
l-*MTP*	1.05 ± 0.40	1.37 ± 0.69	1.48 ± 0.56	1.35 ± 0.40	0.674	0.343	0.307
l-*PLA2*	1.28 ± 0.93	1.33 ± 0.75	1.13 ± 0.31	1.17 ± 0.29	0.874	0.549	0.975

Fish oil (FO), blend of vegetable oils (VO); carbohydrate content: 0 % (CH−) or 20 % (CH+) gelatinized maize starch

Considering FOCH- group as control, relative fold difference between treatments are presented as mean ± SD ($n = 6$) and were analyzed using two-way ANOVA

ApoA1 apolipoprotein A1, *ApoB* apolipoprotein B, *DGAT* diacylglycerol acyltransferase, *FABP* fatty acid-binding protein, *GPAT* glycerol-3-phosphate acyltransferase, *LCAT* lecithin-cholesterol acyltransferase, *LPL* lipoprotein lipase, *MTP* microsomal triglyceride transfer protein, *PLA2* phospholipase A2

* Differences were considered statistically significant at $p < 0.05$

intake also promoted a decrease in LDL-C. On the contrary, dietary carbohydrate promoted an increase in serum HDL-C but only in the FO group. Dietary VO induced a decrease of serum HDL-C (CH × LS interaction).

Data on the expression of key genes encoding proteins involved in lipid digestion (*phospholipase A2, PLA2*), lipid transport (*fatty acid-binding protein, FABP and FABP2*), TAG and PL resynthesis (*glycerol-3-phosphate acyltransferase, GPAT; diacylglycerol acyltransferase, DGAT*), lipoprotein assembly (*apolipoproteins B, A1: ApoB, ApoA1; microsomal triglyceride transfer protein, MTP*) and lipolysis (*lecithin-cholesterol acyltransferase, LCAT; lipoprotein lipase, LPL*) in liver and intestine are presented in Tables 6 and 7, respectively. In the liver, only *LCAT* was affected by diet composition (CH × LS interaction). Hepatic *LCAT* transcript levels were higher in the VO group than in the FO group when fish were fed the diet with no starch. On the other hand, in fish fed the VO diets higher hepatic transcript levels were recorded in CH− than in CH+ groups (CH × LS interaction). In the intestine, *APOA1, FABP2* and *DGAT* transcripts were modulated by dietary carbohydrate but not by lipid source. Intestinal transcript levels of *APOA1, FABP2* and *DGAT* were lower in fish fed the carbohydrate diets.

Discussion

The effect of dietary lipid source and carbohydrate content are discussed separately below as very few interactions between both factors were recorded.

Replacement of FO by a VO blend in the diets induced a slight but significant decrease in apparent digestibility of lipids, which could be related to differences in the dietary

Table 7 Relative mRNA abundance of selected proteins involved in lipid digestion (*PLA2*), lipid transport (*FABP*), TAG and PL resynthesis (*DGAT, GPAT*), lipoprotein assembly (*ApoB, ApoA1, MTP*) and lipolysis (*LCAT, LPL*) in the intestine of gilthead sea bream fed the experimental diets

Lipid source (LS)	Experimental diets				p value*		
Carbohydrates (CH)	FO		VO				
	CH−	CH+	CH−	CH+	CH	LS	CH × LS
i-*ApoA1*	1.03 ± 0.18	0.80 ± 0.21	1.20 ± 0.17	0.93 ± 0.23	0.007	0.086	0.843
i-*ApoB*	1.12 ± 0.50	1.23 ± 0.56	1.70 ± 0.97	1.43 ± 0.90	0.814	0.227	0.548
i-*DGAT*	1.02 ± 0.26	0.82 ± 0.45	1.48 ± 0.48	0.93 ± 0.35	0.033	0.091	0.300
i-*FABP*	1.42 ± 1.04	1.08 ± 1.03	0.72 ± 0.95	1.57 ± 1.22	0.573	0.814	0.204
i-*FABP2*	1.08 ± 0.39	0.53 ± 0.15	1.16 ± 0.52	0.63 ± 0.14	0.001	0.528	0.933
i-*GPAT*	1.08 ± 0.56	1.08 ± 0.37	1.58 ± 0.66	1.52 ± 0.67	0.896	0.068	0.896
i-*LCAT*	1.42 ± 1.36	1.33 ± 1.52	2.68 ± 2.08	2.38 ± 1.83	0.792	0.120	0.882
i-*LPL*	1.12 ± 0.53	0.85 ± 0.29	0.82 ± 0.31	0.72 ± 0.28	0.247	0.181	0.604
i-*MTP*	1.05 ± 0.40	0.98 ± 0.48	1.40 ± 0.20	1.15 ± 0.46	0.365	0.146	0.597
i-*PLA2*	2.55 ± 3.97	1.50 ± 0.55	1.36 ± 1.52	1.82 ± 1.62	0.764	0.658	0.448

Fish oil (FO), blend of vegetable oils (VO); carbohydrate content: 0 % (CH−) or 20 % (CH+) gelatinized maize starch

Considering FOCH− group as control, relative fold difference between treatments are presented as mean ± SD ($n = 6$) and were analyzed using two-way ANOVA

ApoA1 apolipoprotein A1, *ApoB* apolipoprotein B, *DGAT* diacylglycerol acyltransferase, *FABP* fatty acid-binding protein, *GPAT* glycerol-3-phosphate acyltransferase, *LCAT* lecithin-cholesterol acyltransferase, *LPL* lipoprotein lipase, *MTP* microsomal triglyceride transfer protein, *PLA2* phospholipase A2

* Differences were considered statistically significant at $p < 0.05$

FA profile. Lipid digestibility is modulated by FA chain length and degree of unsaturation [45], with digestibility decreasing with FA chain length and increasing with FA unsaturation. This is in accordance with the FA composition of diets in the present study, as FO replacement by VO blend led to an increase of n-6 PUFA and a decrease of the unsaturation index. Further, FA digestibility decreases when the position of the first double bond moves from the methyl end of the carbon chain [18] and the n-3/n-6 PUFA ratio was lower in the VO diets, which may also contribute to explain the lower lipid digestibility of these diets. As stated by Francis *et al.* [18] the lower lipid digestibility of VO diets can be related not to just a single factor but to several dependent factors that are known to affect FA digestibility such as the ones linked to emulsification, enzymatic hydrolysis, lipase specificities and micellar incorporation of the FA.

Although dietary FA profile affected lipid digestibility, post-prandial changes in serum TAG levels, which are the main lipid class in diets, followed a similar kinetic in fish fed the different diets. Postprandial TAG peaked within 6 and 8 h post-feeding, which is in the range of peak times reported for other fish species such as common carp (*Cyprinus carpio*) [46], Senegalese sole (*Solea senegalensis*) [16] and European sea bass [5]. This more pronounced phase in serum TAG concentrations is possibly indicative of appearance of TAG-rich chylomicrons in the circulation. PL along with TAG are two important lipid components in lipoprotein assembly and export of the cell, the former being crucial for lipoprotein export [47]. Contrary to previous observations in European sea bass [5], the serum PL profile over time differed from that of serum TAG but, as for TAG, serum PL concentration was also little affected by the dietary lipid source.

Resynthesis of TAG and PL, a key step in lipoprotein assembly, is thought to be a selective process in fish based mainly on the FA unsaturation degree [4]. Indeed, higher specificities for C18 PUFA to be resynthesized into TAG, and of LC-PUFA to be resynthesized into PL were reported in rainbow trout and in gilthead sea bream [48]. Previously, it was shown *in vitro* with gilthead sea bream intestinal microsomes that 60 % substitution of FO by rapeseed oil had no effects on PL resynthesis but induced a reduction in TAG resynthesis in both glycerol-3-phosphate (G3P) and monoacylglycerol pathways, the two pathways involved in TAG and PL resynthesis [49, 50]. Even though, the lack of alterations of serum PL and TAG concentrations in the present study is in accordance with an absence of intestinal and hepatic regulation of *DGAT* and *GPAT*, two enzymes involved in TAG and PL resynthesis. Similarly, lack of effects on *DGAT* activity [28, 51] or gene expression [5] were reported in other studies in fish with dietary replacement of FO by VO.

The dietary FA profile was demonstrated to affect serum C and LDL-C in humans [52, 53]. In the present study, a lower serum total C, LDL-C (only in the CH + diet) and HDL- C were observed in fish fed the VO diets. Similarly, replacement of FO by VO was reported to decrease serum

total C or LDL and LDL-C in rainbow trout [25] and in Atlantic salmon [22, 23]. Low dietary C levels and the presence of phytosterols in VO diets were linked to these effects. In this study, in the CH- groups C content was slightly higher in fish fed the FO than the VO diet (0.7 % against 0.5 %) but such difference was not observed in the CH+ diets. Thus, we assume that a difference in dietary C was not the main cause for the present results.

Additionally, in previous studies where FO was substituted by VO but diets had the same C levels, plasma C concentration did not change [5, 54]. In this sense, the presence of phytosterols in VO diets (not measured in the present study) might explain our results. Phytosterols are abundant in VO and are structurally and functionally similar to C in animals. Their ability to lower plasma cholesterol and LDL-C has been documented both in humans [55–58] and in fish [59]. The hypocholesterolemic effect of phytosterols is thought to be promoted by an impairment of intestinal C absorption through modulation of C transporters uptake-capacity, competition for C transporters, and decreased incorporation of dietary and biliary C into micelles [55, 56, 60].

The capacity of C biosynthesis at the hepatic level was demonstrated in several fish species, such as Atlantic salmon [29] rainbow trout [61] and European sea bass [21]. However, under VO challenge, both negative [61] and positive [21, 29] regulation of the transcript levels of proteins involved in C biosynthesis were reported. The up-regulation of C biosynthesis induced no major changes of hepatic C content [21, 29] or serum C levels [21] in fish fed VO or FO based diets. This suggests that hepatic C upregulation might be enough to counteract a deficit in absorbed C.

C, either diet derived or synthesized by the liver, is transported in the serum from the liver to extra-hepatic tissues by LDL lipoproteins, which in turn are derived from TAG-rich VLDL by sequential lipolysis mediated by *LPL* [4, 12]. Thus, increased uptake of plasma LDL-C by peripheral tissue cannot be excluded as the cause of reduced serum LDL-C concentration in VOCH+ group. Indeed, increased uptake of serum LDL by peripheral tissues due to up-regulation of *LDL receptors* (*LDL-r*) or increased expression and activity of *LPL* in fish fed VO based diets was advanced as cause for the lower serum lipid and LDL observed in Atlantic salmon fed VO diets compared to FO diets [22]. In another study with the same species fed a VO diet, no dietary regulation of hepatic *LDL-r* transcripts was reported; however, one of the three transcripts for *LPL* were up-regulated in the fat line of Atlantic salmon [23]. In the present study, *LPL* expression was not nutritionally regulated either in the liver or the intestine. Contrarily, up-regulation of *LPL* transcripts were reported in turbot (*Scophthalmus maximus*) fed VO based diets [62].

In the present study, despite the decreased serum HDL-HDL-C concentration in fish fed the VO diets, no dietary regulation of hepatic and intestinal transcript levels of *ApoA1* were observed. VO diets induced an up-regulation of liver *LCAT* transcript levels, but only in the CH- group. *ApoA1* and LCAT are two important components involved in HDL metabolism, especially in the reverse cholesterol transport pathway [63]. *ApoA1* is the main structural protein of HDL and the most potent activator of *LCAT*. *LCAT* is synthesized and secreted primarily by the liver and reversibly binds to lipoproteins, mainly on HDL, and catalyzes the esterification of free cholesterol into cholesteryl ester (CE) [64, 65]. *LCAT* also catalyzes the transfer of FA from phosphatidylcholine in the reesterification of free cholesterol to CE. Thus, transcript levels of *LCAT* may also be regulated by the nature of PLs and FA available for esterification [64, 65].

Changes in HDL-C are expected to be in part mediated by changes in *LCAT* and *ApoA1* concentrations. However, the underlying mechanisms responsible for regulation of serum HDL by *apoA1* and *LCAT* are not yet completely elucidated in mammals [63, 66] and unknown in fish. Indeed, hepatic overexpression of *ApoA1* was reported in Atlantic salmon fed VO-based diets, although differences on serum HDL-C were not observed [23]. In European sea bass fed plant-based diets hepatic overexpression of *ApoA1* was also reported [27]. Although the authors suggested that reverse transport of cholesterol was induced, no data on serum HDL or HDL-C concentrations was provided to support that assumption. Even though our data may suggest an induction of cholesterol transport from tissues to the liver in VOCH- group, no positive variation of serum HDL-C concentration was observed. So, as for LDL-C, the involvement of other mechanisms to explain the decreased serum HDL-C concentration in fish fed the VO diets, namely those related to HDL catabolism (HDL uptake/clearance), cannot be discarded.

Lipid metabolism is reported to be highly modulated by dietary carbohydrates or glucose load [8, 17, 33, 67–69]. Accordingly, changes on postprandial triacylglycerolemia [8], phospholipedemia [21] and cholesterolemia [17, 21] promoted by dietary carbohydrates were described in carnivorous fish.

Hypertriacylglycerolemia is normally associated with the intake of carbohydrate-rich diets in humans [70, 71] and in fish it was reported in rainbow trout [17]. Several mechanisms may be involved in the induction of hypertriacylglycerolemia by dietary carbohydrates, such as mobilization and utilization of stored TAG, an increase in lipid synthesis or a decrease in TAG clearance. In a previous study with gilthead sea bream, serum TAG peaked 1 h after an intramuscular load of glucose, and that peak was associated with increased catabolism of stored body

reserves [69]. On the contrary, in the present study lower serum TAG was observed 6 h post-feeding in fish fed the CH + diets. A large body of evidence indicates that TAG production, and consequently lipoprotein formation and secretion, are driven to a large extent by lipid substrate availability [2, 4]. Thus, the lower serum TAG concentration observed 6 h after feeding (absorptive phase) may be related to a reduced intestinal lipid uptake in the CH+ groups, as corroborated by the decreased transcript levels of key chylomicron assembly proteins (*FABP2, ApoA1, DGAT*) in the intestine but not in the liver. As the experimental diets were isolipidic, it may be assumed that changes in serum TAG and of intestinal transcript of proteins involved in chylomicron assembly are an effect of dietary carbohydrates on lipid digestibility, as described in several fish species, including gilthead sea bream [19, 72, 73]. However, as in the present study no differences in lipid digestibility were observed, the delay of TAG peaks in the CH+ groups (8 h post-feeding) as compared to the CH− groups (6 h post-feeding) could be related to a delay in lipid digestion or absorption induced by dietary carbohydrates. On the contrary, higher serum TAG concentrations were observed at 12 h post-feeding in the CH+ groups than in the CH− groups. At that time, most of the TAG in circulation are of endogenous origin, suggesting that dietary carbohydrate intake might have induced *de novo* lipid synthesis and mobilization of stored TAG. It would be of interest to have data on gene expression of key proteins involved in lipogenesis, lipolysis and lipid uptake at 12 h post-feeding, to confirm this hypothesis.

In European sea bass, in a trial similar to the present one, increased serum C at 18 h after feeding in CH + groups matched the hepatic up-regulation of transcript levels of *HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme reductase*), a key protein involved in C biosynthesis. However, such induction apparently did not occur in the present study.

In contrast to TAG, which are exclusively transported via the chylomicron pathway, C across the intestinal epithelial cells may occur via the chylomicron pathway or the *apoB*-independent pathway. Although *ApoA1* is needed in both pathways, it is a key driver in the *apoB*-independent pathway [74, 75]. Thus, though the decreased transcript levels of intestinal *ApoA1* in CH + group may be linked to slower lipid absorption, mainly in the form of TAG, it is also possible that intestinal uptake or transport of C might also have been affected, particularly in fish fed the VO diets, as serum total C was only lower in fish feeding VOCH + at 6 h after feeding. This may be explained by an increased C-lowering effect of VO in the VOCH + group but further investigation is required to confirm this hypothesis.

Serum PL concentration was reported to be induced by carbohydrate rich diets in European sea bass [21]. Similar results were observed in the present study with gilthead sea bream, but only at 12 h post-feeding. Glucose is an important carbon source for FA synthesis but also for G3P synthesis [76]. G3P is a precursor for the synthesis of both TAG and PL in mammals but the relative contribution of G3P pathway for PL synthesis in fish is still a matter of debate [50, 51]. Although changes in the transcript levels of hepatic and intestinal *GPAT*, an enzyme involved in the first committed step of the G3P pathway, were not observed in the present study, it is possible that the rate of G3P synthesis from glucose metabolism in the fish fed the CH+ diets was adequate for PL synthesis.

Lipid digestibility, serum total C and HDL-C were negatively affected by the inclusion of VO in diets for gilthead seam bream juveniles, probably due to differences in the dietary FA profile and the presence of phystosterols in VO, but no changes were observed at the transcriptional level on lipid absorption and transport mechanisms. On the other hand, dietary carbohydrates seem to modulate transcriptional mechanisms involved in lipid absorption and transport at intestinal level, probably by interfering with TAG availability and by delaying lipid absorption. Considering the reduction in dietary C content with the progressive substitution of FM and FO with vegetable feedstuffs, attention should be paid in the regulation mechanisms of C metabolism, as present data suggests that an interaction between dietary VO and carbohydrates exist that may affect C absorption and transport.

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