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1 **Impact of dietary starch on extrahepatic tissue lipid metabolism in farmed**  
2 **European (*Dicentrarchus labrax*) and Asian seabass (*Lates calcarifer*)**

3

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25

26 **Abbreviation list**

27 <sup>2</sup>H deuterium; <sup>2</sup>H<sub>2</sub>O deuterated water; ACLY, ATP citrate lyase; ACO, acyl-CoA  
28 oxidase; CHO carbohydrates; CPT1, carnitine palmitoyl transferase I; DHAP,  
29 dihydroxyacetone phosphate; DNL, *de novo* lipogenesis; FA, fatty acids; FAS, fatty  
30 acid synthase; G3P, glyceraldehyde 3-phosphate; G6PDH, glucose-6-phosphate  
31 dehydrogenase; DNL, *de novo* lipogenesis; HOAD, 3-hydroxyacyl-CoA  
32 dehydrogenase; LPL, lipoprotein lipase; NMR, nuclear magnetic resonance; PFK, 6-  
33 phosphofructokinase; PK, pyruvate kinase; TAG, triacylglycerol; VAT, visceral  
34 adipose tissue

35 **Abstract**

36 In aquaculture, there is high interest in substituting marine-derived with vegetable-  
37 based ingredients as energy source. Farmed carnivorous fish under high carbohydrate  
38 diets tend to increase adiposity but it remains unclear if this happens by increased  
39 lipid retention/accumulation, promotion of lipogenic pathways, or both. In order to  
40 determine the response of extrahepatic tissue to dietary starch, European  
41 (*Dicentrarchus labrax*) and Asian (*Lates calcarifer*) seabass were fed a control (low  
42 starch; LS) or experimental (high starch; HS) diet, for at least 21 days and then  
43 transferred for 6 days to saltwater enriched with deuterated water  $^2\text{H}_2\text{O}$ . Incorporation  
44 of  $^2\text{H}$ -labelling follows well-defined metabolic steps, and analysis of triacylglycerols  
45 (TAG)  $^2\text{H}$ -enrichment by  $^2\text{H}$ -NMR allowed evaluation of *de novo* lipogenesis (DNL)  
46 in muscle and visceral adipose tissue (VAT). Fractional synthetic rates for TAG-  
47 bound fatty acids and glycerol were quantified separately providing a detailed  
48 lipogenic profile. The FA profile differed substantially between muscle and VAT in  
49 both species, but their lipogenic fluxes revealed even greater differences. In European  
50 seabass, HS promoted DNL of TAG-bound FA, in muscle and VAT. High  $^2\text{H}$ -  
51 enrichment also found in muscle TAG-bound glycerol was indicative of its role on  
52 lipid cycling. In Asian seabass, HS had no effect on muscle FA composition and  
53 lipogenic flux, with no  $^2\text{H}$ -enriched TAG being detected. VAT on the other hand  
54 revealed a strong enhancement of DNL in HS-fed fish along with high TAG-bound  
55 glycerol cycling. This study consolidated the use of  $^2\text{H}_2\text{O}$  as tracer for fish lipid  
56 metabolism in different tissues, under different dietary conditions and suitable to use  
57 in different fish models.

58

59 **Key words:** seabass, barramundi, muscle, visceral adipose tissue, NMR,  
60 triacylglycerol, fatty acids, glycerol.

## 61 **Introduction**

62 As the farming of carnivorous fish species such as seabass continues to grow  
63 worldwide, the industry struggles to find affordable source ingredients that are able to  
64 provide a balanced fatty acid (FA) profile. This should preferably occur while  
65 reducing overall dependence on fishmeal with plant-based ingredients, and as  
66 consequence, increasing dietary carbohydrates (CHO) such as starch. This has been  
67 the case for European seabass *Dicentrarchus labrax* (Messina et al., 2013; Moreira et  
68 al., 2008; Viegas et al., 2016) and Asian seabass (also known as, and hereafter  
69 referred to as barramundi) *Lates calcarifer* (Glencross et al., 2016; Glencross et al.,  
70 2012; Salini et al., 2016). In this context, balanced formulations should be able to  
71 maintain growth performances and yields on the one hand, and provide a final product  
72 that delivers optimal levels of critical nutrients for human consumption on the other.

73 From tissue composition, somatic indices and circulating lipids, feed formulations  
74 with higher CHO content have been considered responsible for an overall increase in  
75 adiposity (Dias et al., 1998; Glencross et al., 2014; Nankervis et al., 2000; Peres and  
76 Oliva-Teles, 2002). However, based on studies addressing the regulation and  
77 transcription of hepatic enzymes (Castro et al., 2015a; Castro et al., 2015b; Dias et al.,  
78 1998; Enes et al., 2006; Glencross et al., 2016), it remains unclear if this happens by  
79 increased lipid retention/accumulation, promotion of lipogenic pathways, or by their  
80 combined effects. Moreover, muscle and visceral adipose tissue (VAT) metabolism,  
81 regarded as preferential lipid storage sites in teleost fish (Weil et al., 2013), are often  
82 overlooked in relation to the liver, as are the factors that control lipid deposition in  
83 these locations. The whole-body fatty acid balance method (Turchini et al., 2008),  
84 already applied in barramundi (Salini et al., 2015) but, to our knowledge not under  
85 high CHO dietary treatments, may be considered as a more holistic analysis.  
86 Nonetheless, this method is still blind to lipid compartmentalization in tissues, both in  
87 terms of content (Castro et al., 2015a; Messina et al., 2013) but also distribution (Wu  
88 et al., 2015).

89 In aquaculture, excess fat deposition in the muscle has been mainly attributed to  
90 environmental and/or nutritional factors that promote lipid retention, such as  
91 temperature (Cordier et al., 2002; Ibarz et al., 2007; Moreira et al., 2008), unbalanced  
92 FA profiles in fish feed (Izquierdo et al., 2003), and starch (Alvarez et al., 1999;  
93 Castro et al., 2015b; Dias et al., 1998; Lanari et al., 1999; Messina et al., 2013),  
94 respectively. As the edible fraction of the fish, this tissue is also under permanent

95 scrutiny by the consumer who associates visible fat depots with fish of “inferior”  
96 quality, especially if compared with wild fish (Grigorakis, 2007; Manthey-Karl et al.,  
97 2016; Vidal et al., 2012). Excess fat deposition around the viscera decreases yields for  
98 producers and appeal for consumers. However, VAT is no longer regarded as a  
99 metabolically inert sink for excess (dietary or synthesized) fat after being associated  
100 in mammalian models with endocrine action, energy homeostasis and metabolic  
101 interplay in several conditions such as obesity and cardiovascular disease (Ronti et al.,  
102 2006). This has spurred recent efforts to study fish adipocyte metabolism and its  
103 response to dietary CHO (Bou et al., 2016) and hormonal treatments, particularly  
104 those closely related to high CHO intake like insulin (Caruso and Sheridan, 2011).  
105 Overall, the capacity of fish to endogenously utilize, accumulate or modify FA, and  
106 then compartmentalize excess fat is still far from being understood. This has been  
107 mostly assessed by tissue composition and indirect interpretation from mRNA  
108 abundance of the enzymatic machinery and transporters involved. In this context the  
109 use of isotopes, both stable and radioactive, has been a useful tool to directly follow  
110 the location and metabolic transformation of dietary substrates. Interesting data on  
111 how extrahepatic tissues handle high CHO levels and its consequences on lipid  
112 metabolism by *in vivo* studies: injection (Brauge et al., 1995) or forced feeding  
113 (Hemre and Storebakken, 2000) of  $^{14}\text{C}$ -glucose; dietary incorporation of  $^{13}\text{C}$ -starch  
114 and subsequent recovery in different tissues (Felip et al., 2012); whole body  
115 conversion to lipids (Ekmann et al., 2013). *In vitro* studies, namely the infusion of  
116 adipocytes with  $^{14}\text{C}$ -glucose (Bou et al., 2016) have also contributed to a better  
117 understanding this subject. Instead of following the metabolic fate of a single labeled  
118 substrate, the stable isotope deuterium ( $^2\text{H}$ ), presented as deuterated water ( $^2\text{H}_2\text{O}$ ) in  
119 fish tanks, rapidly equilibrates with fish body water (Viegas et al., 2011) and gets  
120 widely incorporated in newly synthesized metabolites such as alanine (Marques et al.,  
121 2016), glucose or glycogen (Viegas et al., 2015). Similarly,  $^2\text{H}$  gets incorporated into  
122 different sites of the triacylglycerol (TAG) molecule following well-defined  
123 metabolic steps from which estimations for *de novo* lipogenesis (DNL) and glycerol  
124 turnover can be derived (Viegas et al., 2016). In order to determine the response of  
125 extrahepatic tissues to dietary starch in terms of lipid metabolism we measured  
126 incorporation of  $^2\text{H}$  into muscle and VAT TAG, in seabass and barramundi farmed in  
127 similar settings and fed a control (low starch) or an experimental (high starch) diet.  
128

129 **Material and Methods**

130 *Diet formulation*

131 For this study two diets were formulated to fulfill the energetic requirements of each  
132 species: one low- (LS) and one high-starch (HS) diet (Table 1). For seabass, HS was  
133 formulated by replacing an inert filler of cellulose without nutritional value for 17.8%  
134 starch from gelatinized pea. For barramundi, HS was formulated by replacing protein  
135 and fat for 32.5% starch from gelatinized wheat. Further details on the formulations  
136 may be found in (Viegas et al., 2015) and (Glencross et al., 2014) for seabass and  
137 barramundi, respectively. Fish of both experiments were acclimated to the diets and  
138 experimental conditions for one week.

139

140 *Fish handling*

141 For European seabass, experimental protocol was the same as described previously  
142 (Viegas et al., 2015). Briefly, European seabass (*D. labrax*) from Tinamenor  
143 (Cantabria, Spain) were transported to the lab (Coimbra, Portugal) and randomly  
144 assigned to 2 different 200 L tanks (32 fish per tank; initial mean body weight of  
145  $21.9 \pm 0.3$  g) of a recirculated system supplied with well-aerated seawater ( $20 \pm 1^\circ\text{C}$ ;  
146  $30 \pm 1\text{‰}$  salinity). Fish were fed twice daily to satiety for more than 21 days the  
147 correspondent diet. Experimental procedures complied with the Guidelines of the  
148 European Union Council (86/609/EU). Similarly, barramundi (*L. calcarifer*) from  
149 Betta Barra fish hatchery (Atherton, QLD, Australia) were transported to the lab  
150 (Bribie Island, QLD, Australia) and randomly assigned to 2 different 200 L tanks (30  
151 fish per tank; initial mean body weight of  $51.4 \pm 0.5$  g) of a recirculated system  
152 supplied with well-aerated seawater ( $28 \pm 1^\circ\text{C}$ ;  $35 \pm 1\text{‰}$  salinity). Fish were fed once  
153 daily to satiety for 21 days the correspondent diet. All experiments were performed in  
154 accordance with the Australian code of practice for the care and use of animals for  
155 scientific purposes and were approval by the CSIRO Animal Ethics Committee  
156 (approval numbers: A8-2010 and A8-2016). No mortality was registered.

157

158 *Fish residence in  $^2\text{H}_2\text{O}$  and sampling*

159 Following the feeding period each group of fish from each species was transferred to  
160 a separate tank of  $^2\text{H}$ -enriched seawater for 6 days. Approximate 3.5-5.0%  $^2\text{H}$ -  
161 enrichment was achieved by the addition of 99%-enriched  $^2\text{H}_2\text{O}$  (seabass: Eurisotop,  
162 France; barramundi: Sigma cat. #151882) and tank water  $^2\text{H}$  enrichment was assessed

163 at the beginning and end of the trials (Viegas et al., 2011). This tank was maintained  
164 with an independent closed filtering system but with similar characteristics to the  
165 tanks used during the feeding period in terms of size, volume of water (200 L),  
166 opacity, filtering material and water parameters. During the 6-day residence in  $^2\text{H}$ -  
167 enriched saltwater, fish were fed once a day (*ad libitum*) and sacrificed 24 h after last  
168 meal on day 5. Fish were anesthetized in a 30 L tank of  $^2\text{H}$ -enriched seawater  
169 (seabass:  $0.1 \text{ g L}^{-1}$  of MS-222; barramundi:  $0.02 \text{ mL L}^{-1}$  of Aqui-S<sup>®</sup>), measured,  
170 weighed and blood was drawn from the caudal vein with heparinized syringes. A  
171  $\sim 100 \mu\text{L}$  aliquot was centrifuged ( $3000\times g$ , 10 min), and plasma was stored at  $-20^\circ\text{C}$   
172 for quantification of body water  $^2\text{H}$  enrichments. Fish were sacrificed (seabass: by  
173 cervical section; barramundi: overdose of Aqui-S<sup>®</sup>); muscle tissue was dissected from  
174 the epaxial quadrant (seabass:  $n=8$ ,  $1.6\pm 0.1 \text{ g}$ ; barramundi:  $n=5$ ,  $1.1\pm 0.1 \text{ g}$ ) and  
175 visceral adipose tissue (VAT) (seabass:  $n=8$ ,  $0.5\pm 0.1 \text{ g}$ ; barramundi:  $n=6$ ,  $0.5\pm 0.1 \text{ g}$ )  
176 was carefully stripped from the peritoneal cavity. Both tissues were weighed, freeze-  
177 clamped with aluminum tongs cooled in liquid nitrogen, pulverized with chilled pestle  
178 and mortar and stored at  $-80^\circ\text{C}$  until further analysis.

179

#### 180 *Sample treatment*

181 Muscle and VAT lipids were extracted from pulverized tissue according to Matyash et  
182 al. (2008) using methyl tert-butyl ether, methanol and water mixture  
183 (MTBE:MeOH:H<sub>2</sub>O, 10:3:2.5;  $20 \text{ mL g}^{-1}$  of tissue) transferred to glass amber vials,  
184 dried under N<sub>2</sub> stream and stored at  $-20^\circ\text{C}$ . Triacylglycerols (TAG) were purified by  
185 solid phase extraction with prepacked 2 g cartridges (Discovery<sup>®</sup> DSC-NH<sub>2</sub> 52641-U,  
186 Supelco) according to Ruiz et al. (2004). Muscle TAG quantifications were performed  
187 in a fully-automated analyzer Miura 200 (I.S.E. S.r.l.; Guidonia, Italy) using its  
188 dedicated reagent kit (ref. A-R0100000901; seabass:  $n=7$ , barramundi:  $n=5$ ).

189

#### 190 *NMR analysis*

191 Tank water (TW) and fish body water (BW)  $^2\text{H}$  enrichments were determined by  $^2\text{H}$   
192 NMR using calibrated acetone and analyzed in duplicates as previously described  
193 (Jones et al., 2001). Tank and plasma water content was assumed to be 96,5% and  
194 92% of total sample, respectively. NMR spectra of TAG samples were obtained at  
195  $25^\circ\text{C}$  with a Bruker Avance III HD system with an UltraShield Plus magnet (11.7 T,  
196  $^1\text{H}$  operating frequency 500 MHz) equipped with a 5-mm  $^2\text{H}$ -selective probe with  $^{19}\text{F}$

197 lock and  $^1\text{H}$ -decoupling coil. TAG were reconstituted in chloroform containing a  
198 pyrazine standard as previously described (Viegas et al., 2016) - representative TAG  
199  $^1\text{H}$  and  $^2\text{H}$  NMR spectra are provided as supplementary material (Figure S1). As  $^1\text{H}$   
200 and  $^2\text{H}$  signals are essentially isochronous, the identity of the  $^2\text{H}$  signals can be  
201 confirmed by matching their chemical shifts with their  $^1\text{H}$  counterparts, meaning that  
202 the respective signal assignments apply to both nuclei (Table S1). As control for the  
203 TAG extraction, a FA/glycerol ratio (should be  $\sim 3$ ) was calculated from the area of all  
204  $\alpha$  protons times 2, divided by TAG-bound glycerol sn1,3 protons (Duarte et al., 2014).  
205 The FA profile (in percentage) for saturated (SFA) and unsaturated fatty acids (UFA),  
206 both poly- (PUFA) and monounsaturated fatty acids (MUFA) were estimated from  $^1\text{H}$   
207 NMR spectra according to Viegas et al., (2016). Positional  $^2\text{H}$ -enrichments were  
208 quantified from the  $^1\text{H}$  and  $^2\text{H}$  NMR spectra by measuring the  $^1\text{H}$  and  $^2\text{H}$  intensities  
209 (areas) of selected signals relative to the  $^1\text{H}$  and  $^2\text{H}$  intensities of a pyrazine standard,  
210 after correction for linoleic acid contribution according to Duarte et al. (2014).  
211 Briefly, during their residence in  $^2\text{H}$ -enriched seawater, newly synthesized FA from  
212 *de novo* lipogenesis incorporate  $^2\text{H}$ -enrichment in the FA terminal methyl group  
213 (signal at 0.80 ppm). The same principle applies to for newly synthesized glycerol,  
214 incorporating  $^2\text{H}$ -enrichment in the sn-1,3 glyceryl site (signals at 4.15 ppm).  
215 Fractional synthetic rates (FSR; in  $\% \text{ d}^{-1}$ ) of newly synthesized TAG-bound FA and  
216 TAG-bound glycerol were estimated by dividing the respective positional  $^2\text{H}$ -  
217 enrichment by the BW.  $^2\text{H}$ -enrichments were calculated after systematic subtraction  
218 of 0.015%, taken as the mean background  $^2\text{H}$ -enrichment. If the values were below  
219 zero, these were considered as 0.0 for FSR calculation purposes. Spectra were  
220 processed by applying exponential multiplication to the free-induction decay ( $^1\text{H}$ : 0.1  
221 Hz;  $^2\text{H}$ : 1.0 Hz). Spectra processing and peak integration was performed using  
222 ACD/NMR Processor Academic Edition from ACD/Labs 12.0 software (Advanced  
223 Chemistry Development, Inc.).

224

### 225 *Statistical analysis*

226 Data are presented as mean  $\pm$  S.E.M. Whenever possible, two-way ANOVA was used  
227 to test main effects of, and interactions between, diet and tissue. In the case of  
228 statistically significant interactions, or in the impossibility to perform a two-way  
229 ANOVA, differences between diets were tested using a Student's two-tailed unpaired



230 t-test. Analyses were performed in GraphPad Prism<sup>®</sup> software (GraphPad Software,  
231 Inc.). Differences were considered statistically significant at  $P < 0.05$ .

232

### 233 **Results**

234 Following extraction and subsequent isolation from other lipid classes by solid phase  
235 extraction, TAG gave well-resolved <sup>1</sup>H NMR spectra. The FA/glycerol ratio was not  
236 affected by diet for any tissue of both species, and was overall consistent with a  
237 successful TAG separation (seabass: n=32, 2.96±0.03; barramundi: n=22, 3.03±0.04;  
238 t-test,  $P > 0.05$ ). Signals from PUFA and MUFA moieties dominated the spectra,  
239 while contributions from SFA were relatively minor. In seabass, TAG-bound FA  
240 composition as categorized by <sup>1</sup>H NMR spectra was tested for effects of diet (LS vs.  
241 HS) and tissue distribution (muscle vs. VAT) (Table 2). All were significant for the  
242 latter, symptomatic of a differential profile where SFA were stored to a higher degree  
243 in VAT than in muscle while the opposite trend was observed regarding UFA. Within  
244 UFA, more PUFA, and consequently also more n-3 were found in muscle than in  
245 VAT. In the case of PUFA and MUFA effects were also observed due to dietary  
246 treatment, with PUFA decreasing, and MUFA increasing with elevated dietary starch.  
247 No interactions between dietary treatment and the analyzed tissues were observed, as  
248 summarized in Table 2. For barramundi, the same lipid composition analysis from <sup>1</sup>H  
249 NMR spectra was performed as shown in Table 3. Due to poor signal-to-noise ratio,  
250 probably resulting from technical problems associated with the lipid extraction and  
251 not to low lipid concentration *per se*, two muscle samples (one per diet) were  
252 considered unsuited for spectral analysis. As in seabass, barramundi lipid composition  
253 revealed differential tissue distribution, with the exception of PUFA. As previously  
254 observed, SFA were stored to a higher degree in VAT, while UFA, mainly driven by  
255 MUFA, were preferentially stored in muscle. None of the lipid species were affected  
256 by the dietary treatment even if PUFA mean values seemed to indicate otherwise.  
257 Again, no interactions between variables were observed (Table 3).

258 As equivalent <sup>1</sup>H and <sup>2</sup>H signals resonate at approximately the same chemical shift,  
259 the observed <sup>2</sup>H NMR signals correspond to specific enriched sites in the TAG  
260 molecule. This enrichment is derived from ~0.015% <sup>2</sup>H natural abundance (mean  
261 background <sup>2</sup>H-enrichment) plus excess enrichment from the metabolic incorporation  
262 of <sup>2</sup>H from the 6-day residence in <sup>2</sup>H<sub>2</sub>O-enriched seawater. Specifically, this  
263 incorporation resulted in peaks corresponding to FA terminal methyl group site (non

264 n-3) (signal at 0.80 ppm) revealing TAG-bound FA synthesized *de novo*, and to the  
265 sn1,3 glyceryl site, revealing newly synthesized or cycled TAG-bound glycerol.  
266 Labeling was not detected in glycerol of seabass VAT (Fig. 1), and in both FA and  
267 glycerol of muscle of barramundi (Fig. 2). For both species, glyceryl FSR were  
268 substantially higher than for FA; ~20-80 times higher in the case of seabass muscle,  
269 and ~2-20 times higher in the case of barramundi VAT.  
270 In seabass (Fig. 1), differences in FA FSR were attributable to both tissue distribution  
271 and diet, being significantly higher in muscle in regard to VAT, and HS in regard to  
272 LS. Glycerol revealed higher FSR when compared to FA FSR and was also  
273 statistically lower in the HS when compared with LS. In barramundi VAT, FA FSR  
274 was significantly higher in fish fed the HS diet while glyceryl FSR on the other hand  
275 was unaffected by diet. Muscle TAG levels did not differ between diets for both  
276 species (seabass: LS  $8.4 \pm 1.2$  vs. HS  $7.4 \pm 0.6$ ; barramundi LS  $1.0 \pm 0.2$  vs. HS  $0.7 \pm 0.1$ ,  
277 in  $\text{g } 100\text{g}^{-1}$  of tissue; t-test  $P > 0.05$ ).

278

## 279 **Discussion**

280 In vertebrates the liver is the centerpiece for metabolic regulation and in teleost fish it  
281 is the main lipogenic organ where key endocrine and nutrient sensing mechanisms  
282 intersect (Conde-Sieira and Soengas, 2017). In similar feeding trials, hepatic TAG  
283 content and FA composition was sensitive to high CHO diets (Castro et al., 2015a;  
284 Lanari et al., 1999; Messina et al., 2013; Peres and Oliva-Teles, 2002; Viegas et al.,  
285 2016). In the present study, muscle and VAT from both species presented different  
286 FA profiles, but only in seabass these profiles were responsive to HS diet, particularly  
287 PUFA and MUFA. CHO-stimulated accumulation of muscle lipids has been  
288 associated with transcriptional response of hepatic FA desaturation and elongation  
289 enzymes in rainbow trout (*Oncorhynchus mykiss*) (Kamalam et al., 2012), and  
290 enhanced metabolic elongation rates in the same seabass analyzed in the present study  
291 (Viegas et al., 2016). Interestingly in barramundi muscle, lack of CHO-stimulated FA  
292 profile alterations may relate to absolute lack of  $^2\text{H}$ -enriched TAG, either in the FA or  
293 glycerol moieties. This suggests that this pool had very limited turnover, either in  
294 terms of net FA replacement, or FA-TAG cycling (this latter process accounting for  
295 the higher enrichment of glycerol compared to FA in other tissues as discussed  
296 ahead). Muscle FA profile in this species has proven to be sensitive to dietary lipid  
297 manipulations (Alhazzaa et al., 2011), but as regard to CHO, further studies should be

298 conducted. Notwithstanding, potential muscle FA profile alterations were often  
299 accompanied by little or no variation in muscle lipid content as verified in seabass  
300 (Boujard et al., 2004; Castro et al., 2015a; Dias et al., 1998; Messina et al., 2013;  
301 Peres and Oliva-Teles, 2002). The same could be indirectly inferred in barramundi by  
302 the fact that head-on-gutted lipid composition, mainly composed by muscle tissue,  
303 was unaffected by HS (Glencross et al., 2014). This is most likely associated with the  
304 poor capacity of muscular tissue, which represents about 50-60% of the whole-body  
305 mass, to effectively clear excess CHO. Rainbow trout is one of the most intensely  
306 studied fish species, and under high CHO diets muscle mRNA levels for GLUT4  
307 remained unaltered (Kamalam et al., 2012; Panserat et al., 2009). After a short-term  
308 administration of insulin, muscle GLUT4 (Polakof et al., 2010b), along with muscle  
309 FAS (Polakof et al., 2010a) mRNA levels decreased. A long-term (chronic) insulin  
310 administration reverted these effects, but regardless of the duration of the insulin  
311 treatment, muscle glycogen always increased significantly (Polakof et al., 2010b). In  
312 the seabass from the present study, incorporation of <sup>2</sup>H into glycosyl units revealed  
313 that hepatic glycogen synthesis via direct pathway was significantly augmented in  
314 HS-fed fish (Viegas et al., 2015), so the same could be expected in muscle tissue. It is  
315 worth noting that in rainbow trout fed with <sup>13</sup>C-labelled dietary starch, the fraction  
316 recovered in muscle tissue was for the most part present in lipids and glycogen.  
317 Interestingly, no differences we observed for both parameters between fish fed  
318 digestible and raw starch (Felip et al., 2012). Regardless of its lipogenic action in the  
319 liver, under normal feeding the effects of insulin in fish muscle seem to ineffectively  
320 regulate CHO disposal, particularly in a species where insulin release was poorly  
321 stimulated by dietary CHO (Enes et al., 2010) like the seabass. There is however  
322 margin for improvement and revision of these metabolic models, particularly in the  
323 context of aquaculture, as incredibly versatile trait-selected fish (e.g. Fat and Lean  
324 lines of rainbow trout) unveiled the combined effects of i) modulation of lipogenesis  
325 in the liver by the mTOR pathway (Skiba-Cassy et al., 2009), ii) modulation of  
326 muscle glycolytic (PK and PFK) (Song et al., 2018) and fatty acid oxidation enzymes  
327 (CPT1, HOAD and ACO) (Jin et al., 2014a), and finally iii) improved response to  
328 insulin (Jin et al., 2014b) to achieve fish able to display higher muscle lipid content.  
329 Contrary to barramundi, considerable levels of <sup>2</sup>H-enriched TAG were detected in  
330 seabass muscle. When compared with the liver (Viegas et al., 2016), CHO-stimulated  
331 DNL and/or incorporation of starch into body lipid (muscle and VAT) was lower.

332 Despite the fact that the labeling was recovered from a single type of lipid, the  
333 synthesis, and therefore the  $^2\text{H}$ -enrichment of the TAG glyceryl backbone and its  
334 three esterified FA, happens independently. FA terminal methyls hydrogens become  
335 enriched in  $^2\text{H}$  by DNL (other TAG fatty acyl hydrogens may also become enriched  
336 by elongation reactions). TAG glyceryl hydrogens become enriched via *de novo*  
337 glycerol-3-phosphate synthesis from glyceraldehyde-3-phosphate (G3P) and exchange  
338 between glycerol-3-phosphate and G3P. Their enrichment is suitably discriminated by  
339  $^2\text{H}$  NMR, a feature that mass-based technologies are unable to do unless additional  
340 sample treatment was performed. Regardless of the dietary treatment and species,  
341 TAG-bound glycerol revealed considerably higher  $^2\text{H}$ -enrichment when compared to  
342 TAG-bound FA. It has been hypothesized that fish maintain high glycerol rates of  
343 appearance by constantly cycling (reesterifying) up to two-thirds of circulating TAG  
344 to free FA (Bernard et al., 1999; Magnoni et al., 2008). During exercise, fish muscle  
345 augments lipoprotein lipase (LPL), critical for lipid mobilization (Magnoni et al.,  
346 2013) but TAG-FA turnover rates were maintained unalterably high. In the absence of  
347 glycerol kinase in muscle, this glycerol cannot be recycled to glycerol-3-phosphate  
348 and reused in TAG cycling, being instead shuttled to the liver. Alternatively, *de novo*  
349 synthesis of glycerol-3-phosphate must occur and as consequence, enrichment of the  
350 triglyceride glyceryl moiety is typically much higher than that of the FA, which also  
351 include FA from dietary (unlabeled) origin. Glucose can be a contributing precursor  
352 to glycerol synthesis (through dihydroxyacetone phosphate; DHAP) via an  
353 abbreviated pathway of glycolysis as confirmed by  $^{13}\text{C}$ - (Rito et al., 2018) and  $^{14}\text{C}$ -  
354 glucose (Walter et al., 2006) tracer studies. Higher levels of circulating (unlabeled)  
355 glucose may putatively explain why, glyceryl FSR was lower in the muscle of seabass  
356 fed with HS diet, as already observed in the liver of these fish (Viegas et al., 2016).  
357 This same study also revealed that despite elevated hepatic TAG levels (LS:  $11.1 \pm 0.2$   
358 and HS:  $35.4 \pm 0.7$  g  $100$  g $^{-1}$  liver; t-test  $P < 0.001$ ), this was not attributable to  
359 increased hepatic DNL. So, it was particularly intriguing to find that in muscle, TAG-  
360 bound FA from DNL were significantly increased in the HS diet, even if muscle TAG  
361 levels remained unaltered (LS:  $8.4 \pm 1.2$  vs. HS:  $7.4 \pm 0.6$  g  $100$  g $^{-1}$  muscle; t-test  $P >$   
362  $0.05$ ). This indicates an independent regulation of muscle TAG synthesis activity  
363 during starch feeding, previously observed for rainbow trout (Brauge et al., 1995) but  
364 whose mechanisms are yet to be addressed.

365 In seabass, CHO-derived overall increased adiposity is driven not only by increased  
366 liver lipid content but also by VAT accumulation (Castro et al., 2015a; Dias et al.,  
367 1998; Peres and Oliva-Teles, 2002). This was not observed in the present experiment,  
368 as perivisceral fat index remained unaltered (LS:  $4.9 \pm 0.2$  vs. HS:  $5.1 \pm 0.2$ ) (Viegas et  
369 al., 2016). In rainbow trout, contrary to documented for muscle, in VAT insulin is  
370 regarded as an important hormone for lipid deposition: i) via its anti-lipolytic effects  
371 (Albalat et al., 2006); ii) through upregulation of ACLY and FAS and iii) by  
372 production of NADPH for lipogenesis (Polakof et al., 2010b). In a species from the  
373 same genus, coho salmon (*O. kisutch*), adipocyte GLUT4 transporter was sensitive to  
374 insulin notwithstanding a lower affinity for glucose compared to the mammalian  
375 homolog (Capilla et al., 2004). In another salmonid species, *in vitro* studies revealed  
376 even though the DNL pathway is active in Atlantic salmon (*Salmo salar*) adipocytes,  
377 rates of conversion of glucose into lipids were relatively low (Bou et al., 2016). It is  
378 worth noting that in salmonids, adipocytes were not nutritionally regulated by CHO  
379 (Figueiredo-Silva et al., 2012; Kamalam et al., 2013), but displayed elevated G6PDH  
380 activity and NADPH levels even in the absence of CHO (Barroso et al., 2001). This  
381 seems not to be the case for seabass and barramundi VAT where TAG-bound FA  
382 synthesis via DNL was significantly higher for HS diet. This was also described in  
383 gilthead seabream (*Sparus aurata*) adipocytes which under high CHO diets, up-  
384 regulated G6PDH expression and FAS also tended to increase (Bou et al., 2014).  
385 Besides a stronger response to insulin (Albalat et al., 2007) through LPL activation,  
386 CHO utilization in this species' VAT was also favored over FA oxidation by  
387 modulation of the PPARs transcription factors (peroxisome proliferator-activated  
388 receptors) (Bou et al., 2014).

389 In seabass, FA FSR was significantly lower in VAT than in muscle but was  
390 nevertheless influenced by diet. Contrary to observed in muscle, TAG-FA cycling did  
391 not occur in VAT as interpreted by the lack of  $^2\text{H}$ -enrichment in TAG-bound glycerol.  
392 In barramundi the opposite seemed to take place; on the one hand, no  $^2\text{H}$ -enrichment  
393 was detected in muscle TAG, and on the other, not only VAT FA were extremely  
394 stimulated by the HS diet, but also high FSR for glycerol were estimated. Similar  
395 findings were obtained from Atlantic salmon (*Salmo salar*) adipocytes incubated with  
396  $^{14}\text{C}$ -glucose. Analysis of TAG revealed 16-fold more  $^{14}\text{C}$  incorporation into glycerol  
397 than FA (Bou et al., 2016). This is well within the range observed in the present study  
398 for barramundi VAT TAG with 19-fold excess  $^2\text{H}$ -enrichment of glycerol over FA

399 during HS feeding. Bou and colleagues proposed that the way glucose stimulates  
400 lipogenesis was by stimulating the pentose phosphate pathway, which in turn  
401 generates G3P precursors for TAG glycerol as well as providing NADPH for  
402 sustaining FA synthesis. Our study provides further evidence for this mechanism, not  
403 only in barramundi adipose tissue but also seabass muscle. It should be noted that  
404 despite our best efforts to provide a framework for comparing both species by using  
405 isoenergetic diets, certain parameters differed. Particularly, the diets for barramundi  
406 were not isoproteic but none was protein limiting as it was ensured that the DP:DE  
407 ratio exceeded the established requirements for this species (Glencross, 2008). The  
408 fact that these were not isolipidic may have alone interfered with overall lipid  
409 metabolism, perhaps with stronger effects in the liver rather than in muscle or VAT.  
410 Nonetheless, the present study has provided further insight into the impact and fate of  
411 dietary starch in extrahepatic tissues of two carnivorous seabass species. The FA  
412 profile differed substantially between muscle and VAT, but their lipogenic fluxes  
413 revealed even greater differences. In seabass, HS promoted DNL of TAG-bound FA,  
414 in muscle and VAT. High <sup>2</sup>H-enrichment found in muscle TAG-bound glycerol was  
415 indicative of its role on lipid cycling. In barramundi, HS had no effect on muscle FA  
416 composition and lipogenic flux, with no <sup>2</sup>H-enriched TAG being detected. VAT on  
417 the other hand revealed a strong enhancement of DNL in HS-fed fish along with high  
418 TAG-bound glycerol cycling.

419 Overall, while the aquaculture industry seeks to further stretch the optimization of fish  
420 feed production, the administration of isotopes will provide deeper insights into the  
421 regulation of lipid storage and oxidation in fish species in response to different  
422 combination of macronutrients. This may be particularly interesting in the  
423 procurement of new species to farm or in the optimization and selection of existing  
424 ones, currently happening by trait preference (Jin et al., 2014b; Song et al., 2018) or  
425 by recurring to the wide spectrum of metabolic responses occurring in nature  
426 (Betancor et al., 2016; Marandel et al., 2018).

427

#### 428 **Disclosures**

429 No conflicts of interest, financial or otherwise, are declared by the authors.

430

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#### 445 **References**

- 446 Albalat, A., Saera-Vila, A., Capilla, E., Gutiérrez, J., Pérez-Sánchez, J., Navarro, I.,  
447 2007. Insulin regulation of lipoprotein lipase (LPL) activity and expression in  
448 gilthead sea bream (*Sparus aurata*). *Comp Biochem Physiol B Biochem Mol Biol*  
449 148, 151-159.
- 450 Albalat, A., Sánchez-Gurmaches, J., Gutiérrez, J., Navarro, I., 2006. Regulation of  
451 lipoprotein lipase activity in rainbow trout (*Oncorhynchus mykiss*) tissues. *Gen*  
452 *Comp Endocrinol* 146, 226-235.
- 453 Alhazzaa, R., Bridle, A.R., Nichols, P.D., Carter, C.G., 2011. Up-regulated  
454 Desaturase and Elongase Gene Expression Promoted Accumulation of  
455 Polyunsaturated Fatty Acid (PUFA) but Not Long-Chain PUFA in Lates calcarifer,  
456 a Tropical Euryhaline Fish, Fed a Stearidonic Acid- and  $\gamma$ -Linoleic Acid-Enriched  
457 Diet. *J Agric Food Chem* 59, 8423-8434.
- 458 Alvarez, M.J., López-Bote, C.J., Diez, A., Corraze, G., Arzel, J., Dias, J., Kaushik,  
459 S.J., Bautista, J.M., 1999. The partial substitution of digestible protein with  
460 gelatinized starch as an energy source reduces susceptibility to lipid oxidation in  
461 rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*) muscle.  
462 *J Anim Sci* 77, 3322-3329.
- 463 Barroso, J.B., Peragón, J., García-Salguero, L., de la Higuera, M., Lupiáñez, J.A.,  
464 2001. Carbohydrate deprivation reduces NADPH-production in fish liver but not in  
465 adipose tissue. *Int J Biochem Cell Biol* 33, 785-796.

466 Bernard, S.F., Reidy, S.P., Zwingelstein, G., Weber, J., 1999. Glycerol and fatty acid  
467 kinetics in rainbow trout: effects of endurance swimming. *J Exp Biol* 202, 279-  
468 288.

469 Betancor, M.B., Olsen, R.E., Solstorm, D., Skulstad, O.F., Tocher, D.R., 2016.  
470 Assessment of a land-locked Atlantic salmon (*Salmo salar* L.) population as a  
471 potential genetic resource with a focus on long-chain polyunsaturated fatty acid  
472 biosynthesis. *Biochim Biophys Acta Mol Cell Biol Lipids* 1861, 227-238.

473 Bou, M., Todorčević, M., Fontanillas, R., Capilla, E., Gutiérrez, J., Navarro, I., 2014.  
474 Adipose tissue and liver metabolic responses to different levels of dietary  
475 carbohydrates in gilthead sea bream (*Sparus aurata*). *Comp Biochem Physiol A*  
476 *Mol Integr Physiol* 175, 72-81.

477 Bou, M., Todorčević, M., Torgersen, J., Škugor, S., Navarro, I., Ruyter, B., 2016. De  
478 novo lipogenesis in Atlantic salmon adipocytes. *Biochim Biophys Acta Gen Subj*  
479 1860, 86-96.

480 Boujard, T., Gélineau, A., Covès, D., Corraze, G., Dutto, G., Gasset, E., Kaushik, S.,  
481 2004. Regulation of feed intake, growth, nutrient and energy utilisation in  
482 European sea bass (*Dicentrarchus labrax*) fed high fat diets. *Aquac* 231, 529-545.

483 Brauge, C., Corraze, G., Médale, F., 1995. Effects of dietary levels of carbohydrate  
484 and lipid on glucose oxidation and lipogenesis from glucose in rainbow trout,  
485 *Oncorhynchus mykiss*, reared in freshwater or in seawater. *Comp Biochem Physiol*  
486 *A Physiol* 111, 117-124.

487 Capilla, E., Díaz, M., Albalat, A., Navarro, I., Pessin, J.E., Keller, K., Planas, J.V.,  
488 2004. Functional characterization of an insulin-responsive glucose transporter  
489 (GLUT4) from fish adipose tissue. *Am J Physiol Endocrinol Metab* 287, E348-  
490 E357.

491 Caruso, M.A., Sheridan, M.A., 2011. New insights into the signaling system and  
492 function of insulin in fish. *Gen Comp Endocrinol* 173, 227-247.

493 Castro, C., Corraze, G., Pérez-Jiménez, A., Larroquet, L., Cluzeaud, M., Panserat, S.,  
494 Oliva-Teles, A., 2015a. Dietary carbohydrate and lipid source affect cholesterol  
495 metabolism of European sea bass (*Dicentrarchus labrax*) juveniles. *Brit J Nutr*  
496 114, 1143-1156.

497 Castro, C., Pérez-Jiménez, A., Coutinho, F., Díaz-Rosales, P., Serra, C.A.d.R.,  
498 Panserat, S., Corraze, G., Peres, H., Oliva-Teles, A., 2015b. Dietary carbohydrate



499 and lipid sources affect differently the oxidative status of European sea bass  
500 (*Dicentrarchus labrax*) juveniles. *Brit J Nutr* 114, 1584-1593.

501 Conde-Sieira, M., Soengas, J.L., 2017. Nutrient Sensing Systems in Fish: Impact on  
502 Food Intake Regulation and Energy Homeostasis. *Front Neurosci* 10, 603-603.

503 Cordier, M., Brichon, G., Weber, J.-M., Zwingelstein, G., 2002. Changes in the fatty  
504 acid composition of phospholipids in tissues of farmed sea bass (*Dicentrarchus*  
505 *labrax*) during an annual cycle. Roles of environmental temperature and salinity.  
506 *Comp Biochem Physiol B Biochem Mol Biol* 133, 281-288.

507 Dias, J., Alvarez, M.J., Diez, A., Arzel, J., Corraze, G., Bautista, J.M., Kaushik, S.J.,  
508 1998. Regulation of hepatic lipogenesis by dietary protein/energy in juvenile  
509 European seabass (*Dicentrarchus labrax*). *Aquac* 161, 169-186.

510 Duarte, J.A.G., Carvalho, F., Pearson, M., Horton, J.D., Browning, J.D., Jones, J.G.,  
511 Burgess, S.C., 2014. A high-fat diet suppresses de novo lipogenesis and  
512 desaturation but not elongation and triglyceride synthesis in mice. *J Lipid Res* 55,  
513 2541-2553.

514 Ekmann, K.S., Dalsgaard, J., Holm, J., Campbell, P.J., Skov, P.V., 2013. Effects of  
515 dietary energy density and digestible protein:energy ratio on de novo lipid  
516 synthesis from dietary protein in gilthead sea bream (*Sparus aurata*) quantified  
517 with stable isotopes. *Brit J Nutr* 110, 1771-1781.

518 Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2006. Effect of normal and waxy  
519 maize starch on growth, food utilization and hepatic glucose metabolism in  
520 European sea bass (*Dicentrarchus labrax*) juveniles. *Comp Biochem Physiol A*  
521 *Mol Integr Physiol* 143, 89-96.

522 Enes, P., Sanchez-Gurmaches, J., Navarro, I., Gutiérrez, J., Oliva-Teles, A., 2010.  
523 Role of insulin and IGF-I on the regulation of glucose metabolism in European sea  
524 bass (*Dicentrarchus labrax*) fed with different dietary carbohydrate levels. *Comp*  
525 *Biochem Physiol A Mol Integr Physiol* 157, 346-353.

526 Felip, O., Ibarz, A., Fernández-Borràs, J., Beltrán, M., Martín-Pérez, M., Planas, J.V.,  
527 Blasco, J., 2012. Tracing metabolic routes of dietary carbohydrate and protein in  
528 rainbow trout (*Oncorhynchus mykiss*) using stable isotopes ([<sup>13</sup>C]starch and  
529 [<sup>15</sup>N]protein): effects of gelatinisation of starches and sustained swimming. *Brit J*  
530 *Nutr* 107, 834-844.

531 Figueiredo-Silva, A.C., Panserat, S., Kaushik, S., Geurden, I., Polakof, S., 2012. High  
532 levels of dietary fat impair glucose homeostasis in rainbow trout. *J Exp Biol* 215,  
533 169-178.

534 Glencross, B., Blyth, D., Irvin, S., Bourne, N., Campet, M., Boisot, P., Wade, N.M.,  
535 2016. An evaluation of the complete replacement of both fishmeal and fish oil in  
536 diets for juvenile Asian seabass, *Lates calcarifer*. *Aquac* 451, 298-309.

537 Glencross, B., Blyth, D., Irvin, S., Bourne, N., Wade, N., 2014. An analysis of the  
538 effects of different dietary macronutrient energy sources on the growth and energy  
539 partitioning by juvenile barramundi, *Lates calcarifer*, reveal a preference for  
540 protein-derived energy. *Aquac Nutr* 20, 583-594.

541 Glencross, B., Blyth, D., Tabrett, S., Bourne, N., Irvin, S., Anderson, M., Fox-Smith,  
542 T., Smullen, R., 2012. An assessment of cereal grains and other starch sources in  
543 diets for barramundi (*Lates calcarifer*) – implications for nutritional and functional  
544 qualities of extruded feeds. *Aquac Nutr* 18, 388-399.

545 Glencross, B.D., 2008. A factorial growth and feed utilization model for barramundi,  
546 *Lates calcarifer* based on Australian production conditions. *Aquac Nutr* 14, 360-  
547 373.

548 Grigorakis, K., 2007. Compositional and organoleptic quality of farmed and wild  
549 gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) and  
550 factors affecting it: A review. *Aquac* 272, 55-75.

551 Hemre, G.I., Storebakken, T., 2000. Tissue and organ distribution of <sup>14</sup>C-activity in  
552 dextrin-adapted Atlantic salmon after oral administration of radiolabelled <sup>14</sup>C1-  
553 glucose. *Aquac Nutr* 6, 229-234.

554 Ibarz, A., Beltrán, M., Fernández-Borràs, J., Gallardo, M.A., Sánchez, J., Blasco, J.,  
555 2007. Alterations in lipid metabolism and use of energy depots of gilthead sea  
556 bream (*Sparus aurata*) at low temperatures. *Aquac* 262, 470-480.

557 Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., Rosenlund,  
558 G., 2003. Dietary lipid sources for seabream and seabass: growth performance,  
559 tissue composition and flesh quality. *Aquac Nutr* 9, 397-407.

560 Jin, J., Médale, F., Kamalam, B.S., Aguirre, P., Véron, V., Panserat, S., 2014a.  
561 Comparison of Glucose and Lipid Metabolic Gene Expressions between Fat and  
562 Lean Lines of Rainbow Trout after a Glucose Load. *PLoS One* 9, e105548.

563 Jin, J., Panserat, S., Kamalam, B.S., Aguirre, P., Véron, V., Médale, F., 2014b. Insulin  
564 regulates lipid and glucose metabolism similarly in two lines of rainbow trout  
565 divergently selected for muscle fat content. *Gen Comp Endocrinol* 204, 49-59.

566 Jones, J.G., Merritt, M., Malloy, C., 2001. Quantifying tracer levels of 2H<sub>2</sub>O  
567 enrichment from microliter amounts of plasma and urine by 2H NMR. *Magn  
568 Reson Med* 45, 156-158.

569 Kamalam, B.S., Medale, F., Kaushik, S., Polakof, S., Skiba-Cassy, S., Panserat, S.,  
570 2012. Regulation of metabolism by dietary carbohydrates in two lines of rainbow  
571 trout divergently selected for muscle fat content. *J Exp Biol* 215, 2567-2578.

572 Kamalam, B.S., Médale, F., Larroquet, L., Corraze, G., Panserat, S., 2013.  
573 Metabolism and Fatty Acid Profile in Fat and Lean Rainbow Trout Lines Fed with  
574 Vegetable Oil: Effect of Carbohydrates. *PLoS One* 8, e76570.

575 Lanari, D., Poli, B.M., Ballestrazzi, R., Lupi, P., D'Agaro, E., Mecatti, M., 1999. The  
576 effects of dietary fat and NFE levels on growing European sea bass (*Dicentrarchus  
577 labrax* L.). Growth rate, body and fillet composition, carcass traits and nutrient  
578 retention efficiency. *Aquac* 179, 351-364.

579 Magnoni, L., Vaillancourt, E., Weber, J.-M., 2008. High resting triacylglycerol  
580 turnover of rainbow trout exceeds the energy requirements of endurance  
581 swimming. *Am J Physiol Regul Integr Comp Physiol* 295, R309-R315.

582 Magnoni, L.J., Crespo, D., Ibarz, A., Blasco, J., Fernández-Borràs, J., Planas, J.V.,  
583 2013. Effects of sustained swimming on the red and white muscle transcriptome of  
584 rainbow trout (*Oncorhynchus mykiss*) fed a carbohydrate-rich diet. *Comp Biochem  
585 Physiol A Mol Integr Physiol* 166, 510-521.

586 Manthey-Karl, M., Lehmann, I., Ostermeyer, U., Schröder, U., 2016. Natural  
587 Chemical Composition of Commercial Fish Species: Characterisation of  
588 Pangasius, Wild and Farmed Turbot and Barramundi. *Foods* 5, 58.

589 Marandel, L., Gaudin, P., Guéraud, F., Glise, S., Herman, A., Plagnes-Juan, E.,  
590 Véron, V., Panserat, S., Labonne, J., 2018. A reassessment of the carnivorous  
591 status of salmonids: Hepatic glucokinase is expressed in wild fish in Kerguelen  
592 Islands. *Sci Total Environ* 612, 276-285.

593 Marques, C., Viegas, F., Rito, J., Jones, J., Viegas, I., 2016. Determination of muscle  
594 protein synthesis rates in fish using 2H<sub>2</sub>O and 2H NMR analysis of alanine. *Anal  
595 Biochem* 509, 111-114.

596 Matyash, V., Liebisch, G., Kurzchalia, T.V., Shevchenko, A., Schwudke, D., 2008.  
597 Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J Lipid*  
598 *Res* 49, 1137-1146.

599 Messina, M., Piccolo, G., Tulli, F., Messina, C.M., Cardinaletti, G., Tibaldi, E., 2013.  
600 Lipid composition and metabolism of European sea bass (*Dicentrarchus labrax* L.)  
601 fed diets containing wheat gluten and legume meals as substitutes for fish meal.  
602 *Aquac* 376–379, 6-14.

603 Moreira, I.S., Peres, H., Couto, A., Enes, P., Oliva-Teles, A., 2008. Temperature and  
604 dietary carbohydrate level effects on performance and metabolic utilisation of diets  
605 in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquac* 274, 153-160.

606 Nankervis, L., Matthews, S.J., Appleford, P., 2000. Effect of dietary non-protein  
607 energy source on growth, nutrient retention and circulating insulin-like growth  
608 factor I and triiodothyronine levels in juvenile barramundi, *Lates calcarifer*. *Aquac*  
609 191, 323-335.

610 Panserat, S., Skiba-Cassy, S., Seiliez, I., Lansard, M., Plagnes-Juan, E., Vachot, C.,  
611 Aguirre, P., Larroquet, L., Chavernac, G., Medale, F., Corraze, G., Kaushik, S.,  
612 Moon, T.W., 2009. Metformin improves postprandial glucose homeostasis in  
613 rainbow trout fed dietary carbohydrates: a link with the induction of hepatic  
614 lipogenic capacities? *Am J Physiol Regul Integr Comp Physiol* 297, R707-R715.

615 Peres, H., Oliva-Teles, A., 2002. Utilization of raw and gelatinized starch by  
616 European sea bass (*Dicentrarchus labrax*) juveniles. *Aquac* 205, 287-299.

617 Polakof, S., Médale, F., Skiba-Cassy, S., Corraze, G., Panserat, S., 2010a. Molecular  
618 regulation of lipid metabolism in liver and muscle of rainbow trout subjected to  
619 acute and chronic insulin treatments. *Domest Anim Endocrinol* 39, 26-33.

620 Polakof, S., Skiba-Cassy, S., Choubert, G., Panserat, S., 2010b. Insulin-induced  
621 hypoglycaemia is co-ordinately regulated by liver and muscle during acute and  
622 chronic insulin stimulation in rainbow trout (*Oncorhynchus mykiss*). *J Exp Biol*  
623 213, 1443-1452.

624 Rito, J., Viegas, I., Pardal, M.A., Metón, I., Baanante, I.V., Jones, J.G., 2018.  
625 Disposition of a Glucose Load into Hepatic Glycogen by Direct and Indirect  
626 Pathways in Juvenile Seabass and Seabream. *Sci Rep* 8, 464.

627 Ronti, T., Lupattelli, G., Mannarino, E., 2006. The endocrine function of adipose  
628 tissue: an update. *Clin Endocrinol* 64, 355-365.

629 Ruiz, J., Antequera, T., Andres, A.I., Petron, M.J., Muriel, E., 2004. Improvement of  
630 a solid phase extraction method for analysis of lipid fractions in muscle foods.  
631 Anal Chim Acta 520, 201-205.

632 Salini, M.J., Turchini, G.M., Wade, N.M., Glencross, B.D., 2015. Rapid effects of  
633 essential fatty acid deficiency on growth and development parameters and  
634 transcription of key fatty acid metabolism genes in juvenile barramundi (*Lates  
635 calcarifer*). Brit J Nutr 114, 1784-1796.

636 Salini, M.J., Wade, N., Bourne, N., Turchini, G.M., Glencross, B.D., 2016. The effect  
637 of marine and non-marine phospholipid rich oils when fed to juvenile barramundi  
638 (*Lates calcarifer*). Aquac 455, 125-135.

639 Skiba-Cassy, S., Lansard, M., Panserat, S., Médale, F., 2009. Rainbow trout  
640 genetically selected for greater muscle fat content display increased activation of  
641 liver TOR signaling and lipogenic gene expression. Am J Physiol Regul Integr  
642 Comp Physiol 297, R1421-R1429.

643 Song, X., Marandel, L., Skiba-Cassy, S., Corraze, G., Dupont-Nivet, M., Quillet, E.,  
644 Geurden, I., Panserat, S., 2018. Regulation by Dietary Carbohydrates of  
645 Intermediary Metabolism in Liver and Muscle of Two Isogenic Lines of Rainbow  
646 Trout. Front Physiol 9.

647 Turchini, G.M., Francis, D.S., De Silva, S.S., 2008. A Whole Body, In Vivo, Fatty  
648 Acid Balance Method to Quantify PUFA Metabolism (Desaturation, Elongation  
649 and Beta-oxidation). Lipids 43, 977-977.

650 Vidal, N.P., Manzanos, M.J., Goicoechea, E., Guillén, M.D., 2012. Quality of farmed  
651 and wild sea bass lipids studied by 1H NMR: Usefulness of this technique for  
652 differentiation on a qualitative and a quantitative basis. Food Chem 135, 1583-  
653 1591.

654 Viegas, I., Jarak, I., Rito, J., Carvalho, R.A., Metón, I., Pardal, M.A., Baanante, I.V.,  
655 Jones, J.G., 2016. Effects of dietary carbohydrate on hepatic *de novo* lipogenesis in  
656 European seabass (*Dicentrarchus labrax* L.). J Lipid Res 57, 1264-1272.

657 Viegas, I., Mendes, V.M., Leston, S., Jarak, I., Carvalho, R.A., Pardal, M.A.,  
658 Manadas, B., Jones, J.G., 2011. Analysis of glucose metabolism in farmed  
659 European sea bass (*Dicentrarchus labrax* L.) using deuterated water. Comp  
660 Biochem Physiol A Mol Integr Physiol 160, 341-347.

661 Viegas, I., Rito, J., Jarak, I., Leston, S., Caballero-Solares, A., Metón, I., Pardal,  
662 M.A., Baanante, I.V., Jones, J.G., 2015. Contribution of dietary starch to hepatic

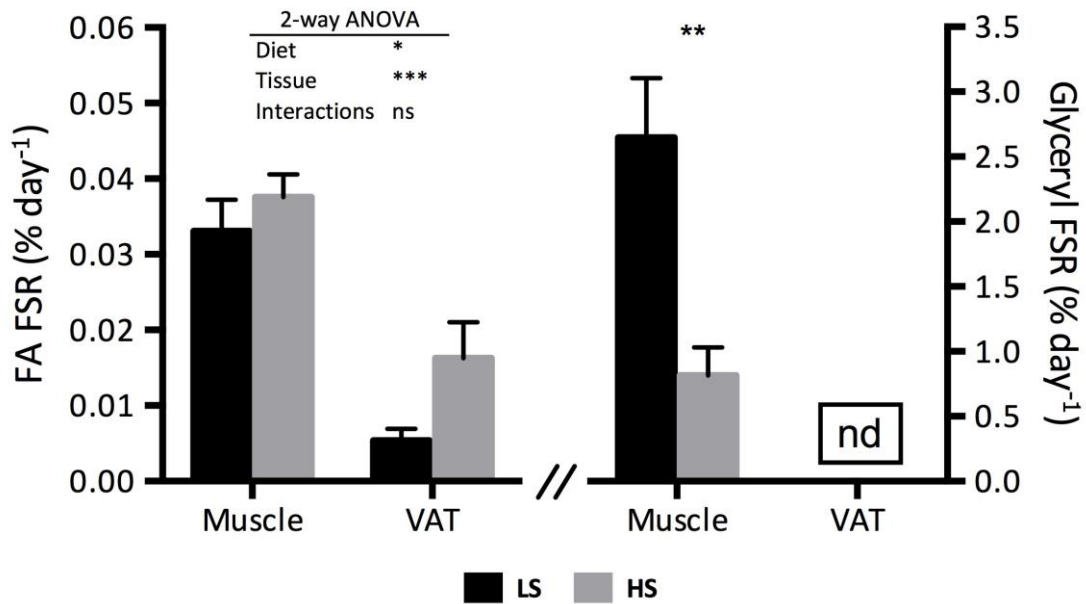
663 and systemic carbohydrate fluxes in European seabass (*Dicentrarchus labrax* L.).  
664 Brit J Nutr 113, 1345-1354.

665 Walter, J.A., Ewart, K.V., Short, C.E., Burton, I.W., Driedzic, W.R., 2006.  
666 Accelerated hepatic glycerol synthesis in rainbow smelt (*Osmerus mordax*) is  
667 fuelled directly by glucose and alanine: a <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance  
668 study. J Exp Zool A Comp Exp Biol 305A, 480-488.

669 Weil, C., Lefèvre, F., Bugeon, J., 2013. Characteristics and metabolism of different  
670 adipose tissues in fish. Rev Fish Biol Fisher 23, 157-173.

671 Wu, J.-L., Zhang, J.-L., Du, X.-X., Shen, Y.-J., Lao, X., Zhang, M.-L., Chen, L.-Q.,  
672 Du, Z.-Y., 2015. Evaluation of the distribution of adipose tissues in fish using  
673 magnetic resonance imaging (MRI). Aquac 448, 112-122.

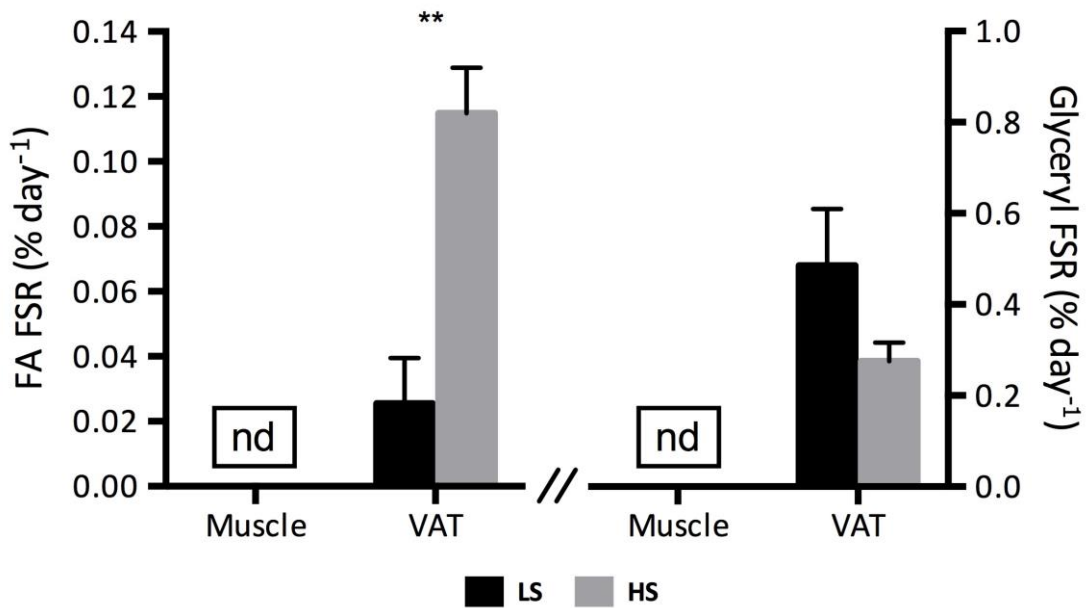
674 **Figure 1**



675

676 **Figure 1:** Fractional synthetic rate for TAG-bound fatty acids (fatty acyls; FA FSR)  
677 and TG-bound glycerol (Glyceryl FSR) expressed as percent of newly synthesized  
678 TAG from *de novo* lipogenesis per day in muscle and visceral adipose tissue (VAT)  
679 of seabass (*D. labrax*) fed with low starch (LS) and high starch (HS) diets, after a 6-  
680 day residence in <sup>2</sup>H-enriched seawater. Mean values ± S.E.M. (n=8) are presented.  
681 When not applicable a two-way ANOVA, significant differences between diets are  
682 tested (t-test) and indicated by asterisks (\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001).

683 **Figure 2**



684

685 **Figure 2:** Fractional synthetic rate for TAG-bound fatty acids (fatty acyls; FA FSR)  
686 and TG-bound glycerol (Glyceryl FSR) expressed as percent of newly synthesized  
687 TAG from *de novo* lipogenesis per day in muscle and visceral adipose tissue (VAT)  
688 of barramundi (*L. calcarifer*) fed with low starch (LS) and high starch (HS) diets,  
689 after a 6-day residence <sup>2</sup>H-enriched seawater. Mean values ± S.E.M. (n=5 for muscle;  
690 n=6 for VAT) are presented. Significant differences between diets are tested (t-test)  
691 and indicated by asterisks (\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001).



692 **Table 1.** Ingredients and proximate composition of the experimental diets provided to  
693 European seabass (*D. labrax*) and barramundi (*L. calcarifer*)  
694

<i>Proximate composition (% dry weight)</i>	Seabass		Barramundi	
	LS	HS	LS	HS
Dry matter	96.0	95.6	93.0	89.0
Crude protein	50.2	50.2	63.3	50.2
Crude fat	16.1	16.1	11.7	6.6
Starch	0.2	17.8	1.6	32.5
Ash	11.5	9.3	9.0	11.5
Gross Energy (kJ g <sup>-1</sup> dry weight)	22.66	22.03	21.3	20.8

695

696 **Table 2.** Percentage of lipid species in triacylglycerol of muscle and visceral adipose  
 697 tissue (VAT) of European seabass (*D. labrax*) fed with a low starch (LS) and high  
 698 starch (HS) diet as determined from <sup>1</sup>H NMR spectra.  
 699

		n-3	PUFA	MUFA	UFA	SFA
Muscle (n=8)	LS	27.6±0.3	46.7±0.9	36.5±1.0	82.3±1.7	17.7±1.7
	HS	27.2±0.3	45.1±0.4	37.3±0.6	82.5±0.6	17.5±0.6
VAT (n=8)	LS	25.5±0.2	40.6±1.1	38.4±0.6	79.1±1.1	21.0±1.1
	HS	24.6±0.5	35.5±2.4	41.8±0.4	77.3±2.2	22.7±2.2
2-way ANOVA	Diet	ns	*	**	ns	ns
	Tissue	***	***	***	*	*
	Interactions	ns	ns	ns	ns	ns

700 Mean values±S.E.M are presented. Two-way ANOVA; sources of variations: diet (LS vs. HS) and  
 701 tissue (muscle vs. visceral adipose tissue) (\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001; ns: not significant).  
 702

703 **Table 3.** Percentage of lipid species in triacylglycerol of muscle and visceral adipose  
 704 tissue (VAT) of Asian seabass (barramundi *L. calcarifer*) fed with a low starch (LS)  
 705 and high starch (HS) diet as determined from <sup>1</sup>H NMR spectra.  
 706

		n-3	PUFA	MUFA	UFA	SFA
Muscle (n=5)	LS	7.8±0.9	26.4±3.7	54.2±3.9	80.7±7.0	19.4±7.0
	HS	10.8±1.8	29.4±3.4	51.5±4.1	73.6±5.8	26.4±5.8
VAT (n=6)	LS	15.1±1.2	26.5±2.5	37.4±0.9	64.0±2.1	36.1±2.1
	HS	16.6±0.2	30.2±0.8	34.4±0.9	63.3±1.1	36.7±1.1
2-way ANOVA	Diet	ns	ns	ns	ns	ns
	Tissue	***	ns	***	**	**
	Interactions	ns	ns	ns	ns	ns

707 Mean values±S.E.M are presented. Two-way ANOVA; sources of variations: diet (LS vs. HS) and  
 708 tissue (muscle vs. visceral adipose tissue) (\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001; ns: not significant).