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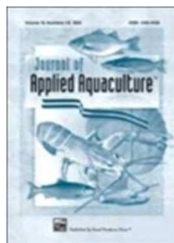

FISH OIL SUBSTITUTION BY SOYBEAN OIL IN Diplodus puntazzo: PERFORMANCE, FATTY ACID PROFILE AND LIVER HISTOLOGY.

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Keywords:	Sharpsnout sea bream, Diplodus puntazzo, Fatty acids, histology, SOYBEAN OIL

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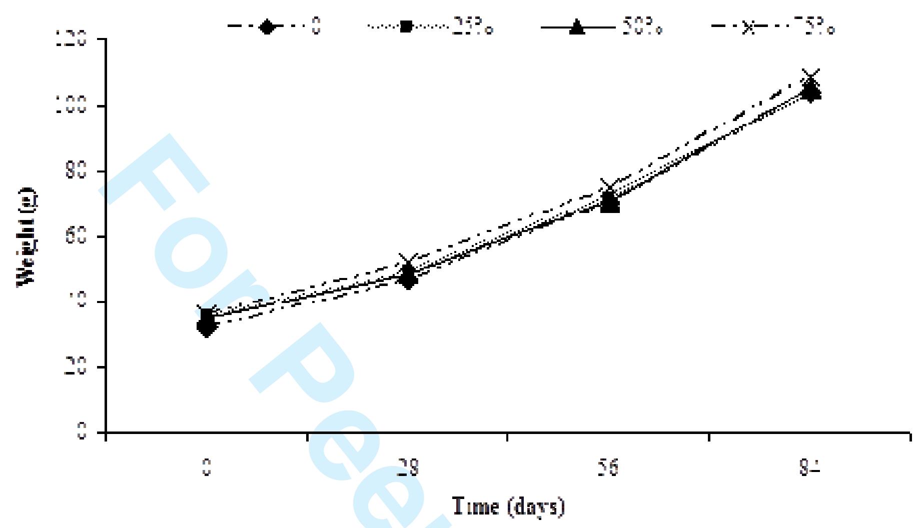

Figure 1. Sharpsnout sea bream growth fed with the four experimental diets.

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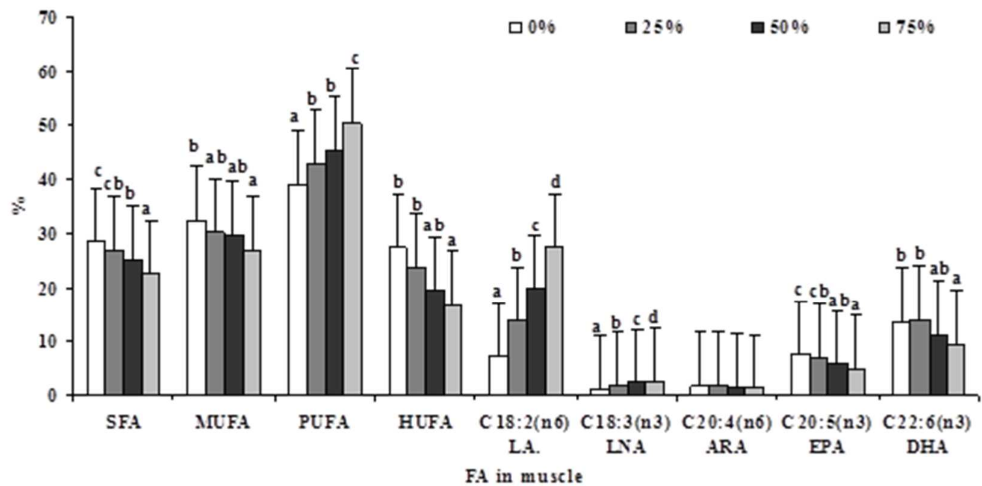

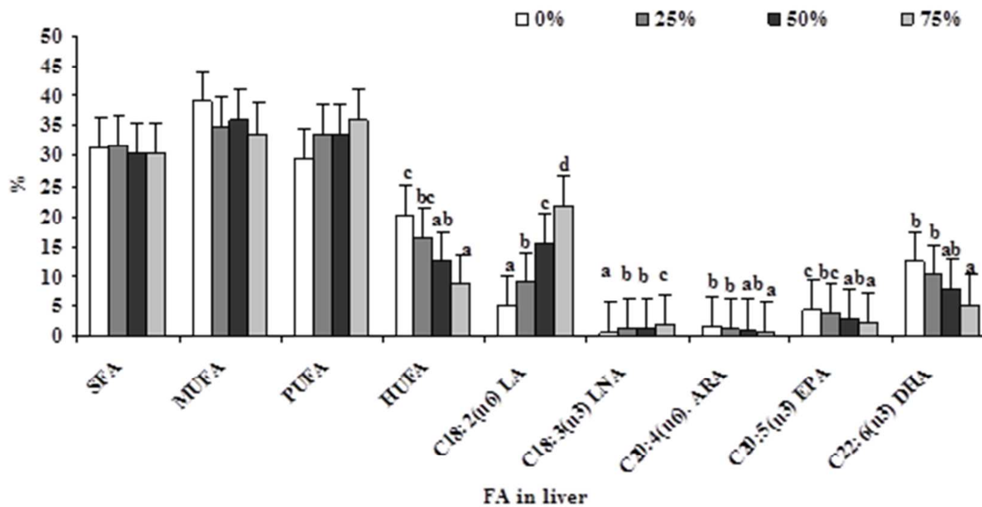

Figure 2. Fatty acids in liver and muscle of Sharpsnout sea bream fed with the experimental diets at the end of the trial. Values expressed in percentage of FAME identified in samples. Data are the means (n = 6) ± SEM. Different letters denote statistical differences $P < 0.05$.

PUFA= (C18:2n6) +(C18:3n3) +(C20:4n6)+(C20:5n3)+(C22:6n3)
n-3 HUFA=(C22:5n3)+(C20:5n3)+(C22:6n3)

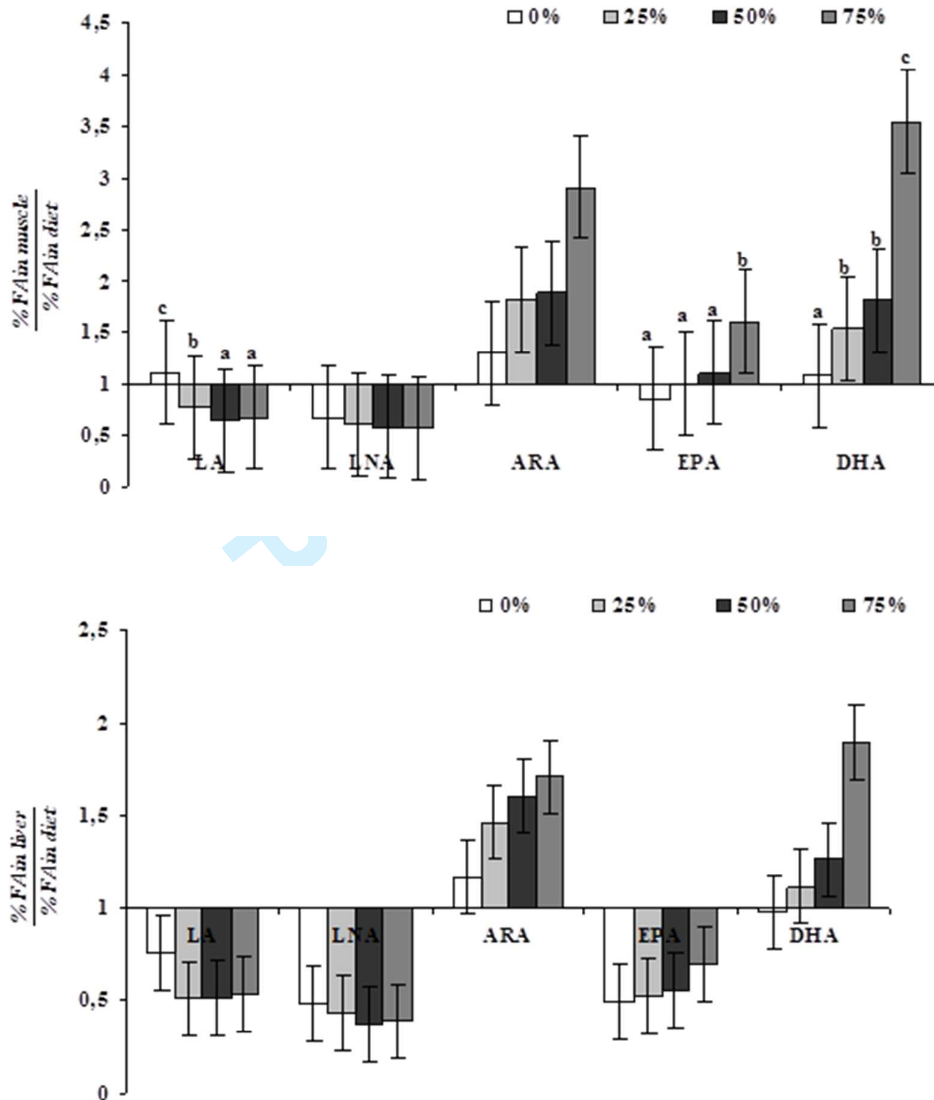

Figure 3. The relation between percentage of fatty acids in muscle (a) and liver (b) of sharpsnout sea bream and experimental diets; Linoleic acids (LA C18: 2n6); Linolenic acids (LNA C18: 3n3), Eicosapentaenoic acids (EPA C20: 5n3) and docosahexaenoic acids (DHA C22: 6n3). Data are the means (n = 6) ± SEM. Different letters denote statistical differences P < 0.05.

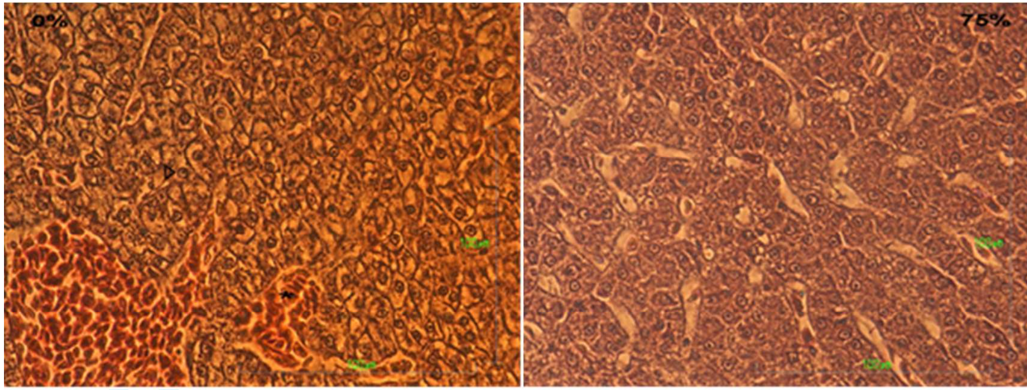

Figure 4. Livers from fish fed the control diet (0%, the left image) and the maximum substitution (75%, the right image). a) hepatocytes, b) blood vessel, c) sinusoids. (Barr size 100μ)

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Table 1 Ingredient contents and proximate composition of diets (dry-weight basis)

Ingredients (g kg^{-1})	0%	25%	50%	75%
Fish meal (5-02-000)[1]	454	454	454	454
Sunflower meal (5-04-739)[2]	235	235	235	235
Wheat (4-05-268)[3]	94	94	94	94
Dextrin (4-08-023)	50	50	50	50
Soybean Oil (4-07-983)	0	50	100	150
Fish oil (7-08-048)	157	107	57	7
Multivitamin mix[4]	6.3	10	10	10
Mineral mix[5]	2.2	2.2	2.2	2.2
Vitamin C	1.5	1.5	1.5	1.5
Proximate composition (% Dry matter)				
Dry matter	93.31	92.97	93.61	92.29
Crude Protein (CP)	41.51	40.98	41.64	41.48
Crude Lipid (CL)	20.62	20.12	21.77	21.41
Ash	10.95	10.40	10.87	10.44
Crude Fibre (CF)	4.85	4.79	4.86	4.85
Values calculated				
Nitrogen free extract (NFE)[6]	22.07	23.71	20.86	21.82
GE (Mj Kg^{-1})[7]	22.01	21.97	22.29	22.28
CP/GE (g MJ^{-1})	18.86	18.66	18.68	18.62

[1]Fish meal: DM: 93.6%; CP: 72.9%; CL: 8.7%; Ash: 17.0%; GE: 20.89 Mj Kg^{-1}
[2]Sunflower meal: DM: 91.4%; CP: 38.3%; CL: 2.2%; CF: 21%; Ash: 8.0% NFE: 30.5%; GE: 15.39 Mj Kg^{-1}
[3]Wheat: DM: 88.5%; CP: 9.1%; CF: 3.2%; Ash: 1.5%; NFE: 84.0%, GE: 17.79 Mj Kg^{-1}
[4]Multivitamin mix (values are g kg^{-1} except those in parentheses): premix, 25; choline, 10; DL-∞-tocopherol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$ 5. Premix composition: retinol acetate, 100000 IU kg^{-1}; calcipherol 500IU kg^{-1}; DL-∞- tocopherol, 10; menadione sodium bisulphite, 0.8; thiamine hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides. 12. (Dibaq-Diproteg)
[5]Zn, 5; Se, 0.02; I 0.5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02. (Dibaq-Diproteg)
[6]NFE (%) = 100- %CP-%CL-%Ash-%CF.
[7]Gross energy: Calculated using: 23.9 kJ g^{-1} proteins, 39.8 kJ g^{-1} lipids and 17.6 kJ g^{-1} carbohydrates

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Table 2. Fatty acids composition of ingredients and experimental diets expressed in % of FAME identified in samples.

	Ingredients		Experimental diets			
	FO	SO	0%	25%	50%	75%
C14:0	4.64	nd	4.98	3.61	2.48	1.57
C15:0	0.51	nd	0.58	0.41	0.24	0.00
C16:0	15.68	10.96	18.20	16.18	14.62	14.50
C18:0	3.76	3.20	4.58	4.29	4.08	4.36
C20:0	0.24	nd	0.26	nd	nd	nd
C22:0	0.17	nd	0.24	0.25	0.34	0.47
C24:0	0.07	nd	0.13	0.14	0.16	0.19
SFA	***24.84***	***14.16***	***28.70***	***24.89***	***21.91***	***21.08***
C16:1	5.79	nd	5.91	4.31	3.02	1.74
18:1n-7	4.32	nd	4.07	3.61	3.31	3.05
C18:1n9c	19.29	25.03	15.78	17.05	19.17	20.32
C20:1	4.85	nd	3.89	2.61	1.54	0.49
C22:1n9	5.08	nd	3.75	2.30	1.45	0.44
C24:1	0.82	nd	0.64	0.53	0.42	0.20
MUFA	***40.16***	***25.03***	***34.04***	***30.41***	***28.91***	***26.24***
C18:2n6c	5.74	54.60	6.38	17.87	30.47	40.63
C18:3n3	2.15	6.15	1.49	2.56	3.63	4.42
C18:3n6	0.19	nd	0.16	0.14	0.11	0.0
C20:2n6	2.07	nd	1.98	0.71	0.95	0.49
C20:3n3	1.20	nd	0.22	0.06	0.09	0.00
C20:3n6	0.20	nd	0.17	0.08	0.07	0.04
C20:4n6	0.94	nd	1.22	0.90	0.68	0.40
C20:5n3	7.87	nd	8.89	6.90	5.18	3.05
C22:2	0.98	nd	0.74	0.55	0.32	0.15
22:5n3	2.25	nd	2.05	1.51	1.05	0.54
C22:6n3	10.44	nd	12.77	9.18	6.17	2.69
PUFA	***34.02***	***60.75***	***36.05***	***40.46***	***48.73***	***52.41***
n-3 HUFA	*20.56*	*0*	*23.70*	*17.60*	*12.40*	*6.27*
n3	23.90	6.15	25.41	20.21	16.13	10.69
n6	9.14	54.60	9.90	19.70	32.29	41.56
n3/n6	2.61	0.11	2.57	1.03	0.50	0.26
EPA/DHA	0.75	0	0.70	0.75	0.84	1.13
n6/n3	0.38	8.88	0.39	0.97	2.00	3.89

nd: not detected.
PUFA= (C18:2n6) +(C18:3n3) +(C20:4n6)+(C20:5n3)+(C22:6n3)
n-3 HUFA=(C22:5n3)+(C20:5n3)+(C22:6n3)

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Table 3. Effect of partial replacement of fish oil by soybean oil on growth and nutritive parameters of sharpsnout sea bream at the end of the trial. Data are mean ± standard Error. Different alphabetic superscripts in the same row indicate significant differences at α = 0.05 (ANOVA)

Treatments	0%	25%	50%	75%	SEM
IBW (g)	32.3	35.3	35.0	36.2	±2.74
FBW (g)	108.4	103.7	104.6	105.7	±2.05
%BWG	221.8	200.2	201.9	200.5	±10.22
SGR[1] (% day^{-1})	1.36	1.31	1.32	1.33	±0.02
TGC[2]	1.88	1.79	1.80	1.82	±0.04
FCR[3]	2.17	2.11	2.28	2.28	±0.12
FI[4]	2.69	2.50	2.66	2.64	±0.13
PER[5]	1.19	1.25	1.13	1.15	±0.06

All values are means of triplicate cases (n= 3).
Initial weight in each phase was considerer as covariable for live weight and SGR.
[1]Specific growth rate (% day^{-1}), SGR = 100 x ln (final weight / initial weight) / days
[2]Thermal Growth Coefficient, TGC=1000*[$Fw^{1/3}$- $Iw^{1/3}$] / (effective Tº)
[3]Feed Conversion ratio, FCR = feed offered (g) / Biomass gain (g)
[4] Feed Intake ratio (g 100 g $fish^{-1}$ day^{-1}), FI = 100 x feed consumption (g) / average biomass (g) x days
[5]Protein efficiency ratio, PER = Biomass gain (g) / protein offered (g)

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Table 4. Effect of soybean oil level on biometric parameters, whole body composition and nutrient retention of sharpsnout sea bream. Data are mean ± standard Error. Different alphabetic superscripts in the same row indicate significant differences at α = 0.05 (ANOVA)

	Initial	0%	25%	50%	75%	SEM
CF[a]		2.18	2.00	2.37	2.12	0.15
HIS[b]		1.44	1.38	1.44	1.41	0.09
MF[c]		2.61	2.20	2.72	2.40	0.26
VSI[d]		9.75	9.02	9.55	9.34	0.57
DP[e]		76.00	76.83	76.49	77.33	0.73
Moisture (%)	70.52	63.56	63.82	63.91	63.09	0.48
CP (% wm)	15.83	16.99	16.59	16.94	16.78	0.30
CL (% wm)	9.54	14.53	15.21	14. 89	15.11	0.49
Ash (% wm)	4.10	3.92	4.14	3.97	4.04	0.13
Calculated values						
GE ($MJ\ kg^{-1}$)[f]	7.58	9.85	10.02	9.97	10.02	0.24
CPE (%)[g]		20.97	21.11	20.00	19.98	1.51
GEE (%)[h]		24.52	26.03	23.96	24.32	1.78

All values are means of triplicate cases (n= 3).
[a] Condition Factor, CF = [body weight (g) / total length (cm) ^{3}] x 100.
[b] Hepatosomatic index, HSI = [liver weight (g)/ body weight (g)] x 100.
[c] Mesenteric fat index, MFI = [mesenteric fat weight (g) /body weight (g)] x 100.
[d] Viscerosomatic index, VSI = 100 x [visceral weight (g)/ fish weight (g)]
[e] Dress out percentage, DP =100 x [total fish weight (g) – visceral weight (g) –head weight (g)]/ fish weight (g)
[f] Gross energy, GE = Calculated using: 23.9 kJ g^{-1} proteins, 39.8 kJ g^{-1} lipids and 17.6 kJ g^{-1} carbohydrates
[g] Crude protein efficiency, CPE (%) = (Increment of protein corporally (g)) x 100 / (ingestion protein,(g))
[h] Grow energy efficiency, GEE (%) = (%) (Increment of energy corporally, (kJ)) x 100 / (ingestion de energy (kJ))

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Table 5. Hepatocyte quantification and morphological evaluation of sharpsnout sea bream liver at the end of the experimental period. Data are mean ± standard Error . Different alphabetic superscripts in the same row indicate significant differences at $\alpha = 0.05$ (ANOVA)

	0%	25%	50%	75%	SEM
Hepatocytes[1]	768	1013	828	833	72.05

	Liver nuclei			Liver hepatocyte cytoplasm			Hepatocyte vacuolation			Pancreatic acinar cells		
	1	***2***	***3***	***1***	***2***	***3***	***1***	***2***	***3***	***1***	***2***	***3***
0%	6	0	-	6	-	-	5	1	-	6	-	-
25%	5	1	-	5	1	-	4	2	-	6	-	-
50%	6	-	-	5	1	-	2	4	-	6	-	-
75%	5	1	-	2	4	-	2	4	-	6	-	-
P-value	0.5708			0.0776			0.2881			0.3975		

1 (Healthy) 2 (Intermediate) 3 (Degraded)
Six replicates per treatment were analysed.
[1] Hepatocytes / 125000µm^2

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FISH OIL SUBSTITUTION BY SOYBEAN OIL IN *Diplodus puntazzo:* PERFORMANCE, FATTY ACID PROFILE AND LIVER HISTOLOGY.

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Keywords: Sharpsnout sea bream, *Diplodus puntazzo*, fatty acids, histology, soybean oil.

Running title: Fish oil substitution by soybean oil in sharpsnout sea bream nutrition

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26 **Abstract.** The present study was performed to determine the effect of soybean oil on
27 the performance and liver histology in sharpsnout sea bream. Four experimental diets
28 were formulated containing 0%, 25%, 50% and 75% of soybean oil substituting of fish
29 oil. Fish weighing 35 g were fed for 84 days. Increasing the level of soybean oil had no
30 significant effects on growth and feed efficiency parameters. Biometrics, body
31 composition, protein- and energy- efficiency were not affected by the fish oil
32 replacement. Muscle and liver fatty acids reflected fish oil substitution. Moreover,
33 histology did not show statistical differences among treatments.
34

35 **Introduction**

36 Sharpsnout sea bream (*Diplodus puntazzo*) is a promising species for Mediterranean
37 aquaculture, although, this fish is already produced in Italy, Greece and Cyprus among
38 others. It has many advantages: it is easy to reproduce and aquacultures farmers can use
39 the same infrastructure used to produce other sparids.

40 On the other hand, fish oil (FO) has been used as a basic component of fish nutrition in
41 aquaculture, but the rising prices of this basic material have prompted the use of
42 alternative oils. In marine fish feed using vegetable oils as single lipid source is limited
43 by the low ability of these species in converting linoleic acid (18:2n - 6) and linolenic
44 acid (18:3n - 3), abundant in many vegetable oils, into arachidonic acid (ARA; 20:4n -
45 6), eicosapentaenoic Acid (EPA; 20:5n - 3) and docosahexaenoic acid (DHA; 22:6n -
46 3),) which are essential for marine fish.

47 The most common vegetal oil used as a fish oil substitution is soybean oil (SO), because
48 it is readily available, often at lower prices than marine oils (Ruyter et al. 2006).
49 Soybean oil has been widely used to feed marine species such as Gilthead sea bream,
50 (Izquierdo et al. 2003; Martínez-Llorens et al. 2007), sea bass (Richard et al. 2006),

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51 black sea bream (Peng et al. 2008) and turbot (Regost et al. 2003), as well as continental
52 fish such as Atlantic salmon (Ruyter et al. 2006; Grisdale-Helland et al. 2002) with
53 different results ranging from optimum response to deleterious effects.

54 Only two trials have been conducted in Diplodus species replacing fish oil with animal
55 fat or vegetal fish (Piedecausa et al. 2007, Nogales- Merida et al. 2011a). Piedecausa et
56 al. (2007) also indicated that sharpsnout sea bream may be able to use dietary vegetable
57 oils in a more efficient manner. Nogales-Merida et al. (2011a) did not obtain significant
58 growth differences, although a tendency to diminish fish growth when pork fat inclusion
59 increased was observed.

60 The inclusion of vegetable oils in diets for fish modifies the fatty acid profile and can
61 significantly affect fillet quality. Especially, reducing the levels of eicosapentaenoic
62 Acid and docosahexaenoic acid, while oleic-, linoleic- and linolenic acid increase. In
63 fact, it has been shown in sharpsnout sea bream studies (Piedecausa et al. 2007,
64 Nogales-Merida et al. 2011a) that the muscle fatty acid composition reflects the profile
65 of the diet fatty acids profile.

66 Llevels of 60% of fish oil replacement during long feeding periods may cause
67 histological alterations in intestine and liver, increasing the deposition of fat (Caballero
68 et al., 2004). In fact, high levels of plant oil as a lipid source have also been previously
69 associated with the degeneration in histological tissue structure (Alexis, 1997), resulting
70 in an accumulation of large lipid vacuoles in the enterocytes and hepatocytes (Olsen et
71 al., 2003; Caballero *et al.* 2002 and Ruyter *et al.* 2006),likely to be due to selective fat
72 accumulation of 18:2n-6 and 18:1n-9 (Ruyter *et al.* 2006). In sharpsnout sea bream,
73 liver nuclei, liver hepatocyte cytoplasm, hepatocyte vacuolation and pancreatic acinar
74 cells remained significantly unchanged when animal fat substituting fish oil was fed
75 (Nogales-Merida et al., 2011a).

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3 76 Thus, the present study aimed at clarifying the effects of partial replacement of fish oil
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5 77 by soybean oil on sharpsnout sea bream, its nutritive utilisation, somatic parameters,
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7 78 body composition, muscle and liver fatty acid profile and liver histology.
8
9 79 **Material and Methods**
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11 80 *Diets*
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13 81 Four extruded isolipidic (21% CL) and isonitrogenous (41% CP) diets were prepared by
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15 82 replacing fish oil with soybean oil (Table 1) at 0%, 25%, 50% or 75% of total lipids.
16
17 83 The four diets will be identified by their SO levels. The increased level of soybean oil in
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19 84 the experimental diets reflected the fatty acid composition of the feed (Table 2). A
20
21 85 gradual increment in linoleic acid (LA) (6.4%, 17.9%, 30.5% and 40.6% respectively),
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23 86 linolenic acid (LNA) (1.5%, 2.6%, 3.6% and 4.4% respectively) could be observed, and,
24
25 87 as a consequence, the sums of the polyunsaturated fatty acids (PUFA) also rose as SO
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27 88 inclusion was increased (36%, 40.5%, 48.7% and 52.4% respectively). However, the
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29 89 high unsaturated fatty acid (n-3 HUFA) values decreased as SO inclusion was increased
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31 90 from 23.70% to 6.27%. A decreasing percentage in DHA, EPA and the n3 / n6 ratio
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33 91 was observed as a consequence of replacing FO by SO. As a result, saturated fatty
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35 92 acids (SFA) diminished, while SO inclusion increased.
36
37 93 In this experiment, fish meal was replaced at 20% by sunflower meal in all diets, as fish
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39 94 growth and feed efficiency were not altered by the inclusion of this vegetal meal
40
41 95 (Nogales-Merida et al., 2010, 2011b).
42
43 96 The diets were prepared with the cooking extrusion process, using a semi-industrial
44
45 97 twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). The processing
46
47 98 conditions were as follows: 0.63g screw speed, a temperature of 110 °C, and a pressure
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49 99 of 40–50 atm. The experimental diets were assayed in triplicate groups. The fish were
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51 100 fed by hand twice a day (9.00 and 16.00) until apparent satiation (to study the possible
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3 101 effects of soybean oil on palatability). The pellets were distributed slowly to allow all
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5 102 fish to eat. The uneaten diet was collected and dried to determine feed intake (FI).
6
7 103 *Growth trial and fish sampling*
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9 104 Fish were transported from University of Valencia, Spain with an average weight of 15
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11 105 g. Prior to the feeding trial, all fish were acclimated to the indoor rearing conditions for
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13 106 4 weeks and fed a standard diet (Microbaq 15 (CP 50%; CL 20%; Nitrogen free extract
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15 107 (NFE) 13% and Ash 10%) (Dibaq Diproteg, Segovia, Spain)). Ten fish were introduced
16
17 108 per each pen. A total of 120 juveniles (average weight 34.8 ± 7.0 g) were distributed in
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19 109 12 pens (three pens per fibre cylindrical tanks of 750 l of capacity). Each pen has a 98 l
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21 110 capacity.
22
23 111 The duration of the trial was 84 d and was conducted in a recirculating marine water
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25 112 system (65 m^3 capacity) with a rotary mechanical filter and a gravity biofilter
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27 113 (approximately 6 m^3). The mean water temperature was22.8±1.3 °C (mean±SD),
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29 114 salinity was 33±1 g l^{-1}, the level of dissolved oxygen was 6.6±0.5 mg l^{-1} and pH ranged
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31 115 from 7.5 to 8, NO^{2-} 0.21 ± 0.1 mg l^{-1}, NH^{4+} 0.0 mg l^{-1} and NO^{3-} 34.1±10.6mg
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33 116 l^{-1}during the trial. All tanks were equipped with aeration. The water was heated by a
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35 117 heat pump installed in the system. The photoperiod was natural, and all tanks had
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37 118 similar light conditions. All fish were weighed at intervals of approximately 30 days..
38
39 119 Prior to weighing, the fish were anaesthetised with clove oil (Guinama®, Valencia,
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41 120 Spain) containing 87% of eugenol. At the end of the growth trial, all fish were
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43 121 individually weighed. Five fish were randomly sampled from each tank and used for the
44
45 122 determination of biometric parameters and for proximate analysis.
46
47 123 The samples from each tank were pooled and stored at −30 °C.
48
49 124 *Proximate composition analyses*
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2
3 125 Chemical analyses of the dietary ingredients were performed prior to diet formulation.
4
5 126 Diets and their ingredients, as well as the whole body were analysed according to the
6
7 127 Association of Official Analytical Chemists (AOAC, 1990) procedures: dry matter
8
9 128 (105º C to constant weight), ash (incinerated at 550ºC to constant weight) and crude
10
11 129 protein (N x 6.25) were determined by a Kjeltec 2300 Auto Analyser, (Tecator,
12
13 130 Höganas, Sweden) and lipid was determined using a Foss Tecator Soxtec 1043
14
15 131 extraction unit using diethyl ether as a solvent. All analyses were performed in
16
17 132 triplicate.
18
19 133 Fatty acid methyl esters (FAMEs) of total lipids were prepared directly as previously
20
21 134 described by O´Fallon, et al. (2007). FAMEs were extracted from raw material,
22
23 135 experimental diets and six subsamples of liver and muscles from each treatment.
24
25 136 FAME analysis was performed in a Focus Gas Chromatograph (Thermo, Milan, Italy)
26
27 137 equipped with a split/splitless injector and a flame ionisation detector. Separation of
28
29 138 methyl esters was performed in a fused silica capillary column SP^{TM} 2560 (Supelco,
30
31 139 PA, USA) (100 m x 0.25 mm x 0.2 µm film thickness). The carrier gas was helium at a
32
33 140 linear velocity of 20 cm sec^{-1}. The samples were injected with a split ratio of 1/100.
34
35 141 The initial oven temperature was set at 140 ºC held for 5 min and increased to 240 at 4
36
37 142 ºC min^{-1} and finally maintained at that temperature for 30 min. Both detector and
38
39 143 injector temperatures were set at 260 ºC. The individual fatty acids were identified by
40
41 144 comparing their retention times with standards of fatty acid methyl esters supplied by
42
43 145 Supelco (PA, USA).
44
45 146 *Histological sampling*
46
47 147 The examination of the liver was conducted using sections of 5 µm cut and stained with
48
49 148 haemoatoxylin and eosin for examination with light microscopy. The morphology of the
50
51 149 liver was evaluated on a scale of 1 to 3 using the criteria of McFadzen et al. (1997).
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150 Grade 1 corresponds to a healthy liver with lightly granular, small and distinct nuclei
151 and hepatocyte cytoplasm with a structure varying in texture and scattered granules with
152 eosin-positive patches. Grade 2 corresponds to an intermediate condition. The nuclei
153 have abundant dark granules, and the nucleoli are enlarged or indistinct. The cytoplasm
154 is homogeneous and is vacuolised only to a very limited degree. Grade 3 indicates a
155 degraded liver with small, dark, pyknotic nuclei. The cytoplasm is hyaline with a lack of
156 texture and with large sinusoidal spaces. The grade of each liver was determinated by
157 eight observations.

158 The quantification of hepatocytes was done per area (125.000 μm^2).

159 *Ethical statement*

160 The *Diplodus puntazzo* study complied with European Union Council Directive
161 2010/63/ UE, which lays down minimum standards for the protection of animals, and
162 was also in accordance with Spanish national legislation (Spanish Royal Decree
163 53/2013) protecting animals used in experimentation and for other scientific purposes.
164 The experimental protocol was approved by the Committee on the Ethics of Polytechnic
165 University of Valencia (UPV).

166 Fish in the tanks were checked on a daily basis. Also, fish were weighed individually
167 every four weeks and their health status was assessed through observation, after
168 sedation with clove oil dissolved in water (1 mg/100 ml of water) to minimise animal
169 suffering. Animals were euthanised by an excess of clove oil (150 mg/l) and then
170 dissected.

171 *Statistical analysis*

172 Growth data and nutritive parameters were treated using multifactor analysis of variance
173 (ANOVA), introducing the initial live weight as covariate (Snedecor & Cochran, 1971).

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3 174 The Newman-Keuls test was used to assess specific differences among diets at 0.05
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5 175 significant levels (Stat graphics, Statistical Graphics System, Version Plus 5.1,
6
7 176 Herndon, Virginia, USA). Hepatocyte quantification was analysed by One-Way
8
9 177 ANOVA and analysis by McFadzen et al. (1997) criteria was done using a cross
10
11 178 tabulation and Chi-Square contrast (X^2) methodology.
12
13
14 179 **Results**
15
16 180 Fish fed with the different experimental diets showed good growth over the 84 days that
17
18 181 the experiment lasted (Figure 1), and fish survival was higher than 94% and without
19
20 182 statistical differences among treatments.
21
22
23 183 Growth parameters and feed efficiency are presented in Table 3. All diets were readily
24
25 184 accepted, and no differences in FI (Feed Intake) were observed. Feed efficiency did not
26
27 185 exhibit statistical differences in diets. An average biomass weight gain (BWG) of 206%
28
29 186 was achieved at the end of the trial. The final body weight fluctuated between
30
31 187 experimental diets from 104 to 108 g and the specific growth rate (SGR) was between
32
33 188 1.3 and 1.6% day^{-1}.
34
35
36 189 Biometric parameters, nutrient utilization and body composition are shown in Table 4.
37
38 190 There were no statistical differences in biometric parameters for fish fed the different
39
40 191 diets. There were no significant differences in muscle composition at the end of the trial
41
42 192 in terms of moisture, ash, crude protein and crude lipid and similar results were
43
44 193 obtained in crude protein efficiency (CPE) and gross energy efficiency (GEE).
45
46
47 194 Muscle fatty acid profiles are presented in Figure 2. Most relevant fatty acids (FA) for
48
49 195 fish growth and health were also affected by FO substitution ($P<0.05$). There were
50
51 196 statistical differences in SFA and fish fed the 75% SO diet had the lowest value
52
53 197 (28.6%). In monounsaturated fatty acids (MUFA), there were also differences, with
54
55 198 fish fed the 75% SO diet, presenting the lowest value (26.8%). In LA, the highest value
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199 obtained was in the 75% SO diet (27.4%) compared with the 0% SO diet (7.1%). The
200 highest LNA value was reported with fish fed the 75% SO diet (2.5%). ARA was the
201 only EFA that did not present statistical differences among treatments. The lowest EPA
202 values were obtained with the 75% and 50% SO diets (4.9% and 5.7%) compared with
203 the 0% and 25% SO diets (7.6% and 6.9%). Similar DHA levels were observed in fish
204 fed the 0% and 25% SO diets (13.8% and 14.1%), being higher than the other two diets.
205 The highest PUFA values were observed in the 75% SO diet (50.5%), but n-3 LCPUFA
206 decreased when SO inclusion increased.
207 In fish liver, the values were similar to those obtained in fish muscle. There were no
208 difference in SFA, ranging from 30.3 to31.4% MUFA were no statistically different and
209 the values fluctuated from 33.7 to 39%. PUFA ranged from 29.6 to 35.9%. LCPUFA
210 values presented statistical differences: fish fed the 50% and 75% SO diet exhibited the
211 lowest values (12.53% and 8.58%) with respect to the 0 and 25% SO diets (20.19% and
212 16.57%). The lowest LA and LNA values were obtained with the 0% SO diet (4.8 and
213 0.7%, respectively). In contrast, lowest ARA, EPA and DHA levels were obtained with
214 the 75% SO diet (0.7% ARA; 2.1% EPA and 5.1% in DHA, respectively).
215 When muscle and liver fatty acids are related with the percentage of fatty acids of their
216 experimental diets, some interesting data can be observed (Figure 3): statistical
217 differences were observed in EPA and DHA (Figure 3A) being lower in fish fed the
218 75% SO diet (1.6% and 3.5%, respectively). Similar results were observed in ARA but
219 without statistical differences. On the contrary, fish fed the control diet reported the
220 highest LA value (1.1%) compared with the other treatments. In Figure 3b, no
221 statistical differences were observed, but the same EFA tendency reported in muscle
222 (3a) was observed in fish liver.

1
2
3 223 Hepatocyte quantification and the morphological status of hepatic cells are reported in
4
5 224 Table 5. Hepatocyte quantification did not present statistical differences among
6
7 225 treatments, being 768, 1013, 828 and 835 Hepatocytes 125000 m^{-2} in the 0, 25, 50 and
8
9 226 75% SO diets. Histological analysis of hepatic cells (liver nuclei; liver hepatocyte
10
11 227 cytoplasm, hepatocyte vacuolation and pancreatic acinar cells) did not exhibit any
12
13 228 statistical differences. Regular shaped hepatocytes with some lipid accumulation in the
14
15 229 cytoplasm and, in most cases, central allocated nuclei were observed in fish fed the
16
17 230 experimental diets (Fig. 4).
18
19
20 231 **Discussion**
21
22
23 232 Total fish oil substitutions by a mixture of oils and / or fats do not affect growth in
24
25 233 marine species (Turchini et al, 2003 and Glencross et al. 2003). The same occurs when
26
27 234 FO is replaced by single oil supplemented with HUFAs (Craig & Gatlin, 1995).
28
29 235 Moreover, when fish oil is replaced to a great extent by a single lipid source, the results
30
31 236 of growth are worse.
32
33
34 237 The results of this experiment indicate that soybean oil does not have a negative effect
35
36 238 on growth performance in *Diplodus puntazzo*. This species has shown a better
37
38 239 acceptance of alternative lipid sources, both of animal (Nogales-Merida et al. 2011a)
39
40 240 and plant origin (Piedecausa et al., 2007) when compared to other sparids or fresh water
41
42 241 fish, possibly due to their omnivorous habits. Piedecausa et al. (2007) obtained good
43
44 242 and similar results with total fish oil substitution with a single oil, which was not tested
45
46 243 in the present work, as these diets, in addition to lipid replacement, also had in their
47
48 244 composition a proportion of plant protein sources. Nogales-Merida et al. (2011a) did not
49
50 245 obtain significant growth differences replacing fish oil by pork fat at the same level than
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52 246 in the present experiment.
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247 With regard to nutritional parameters, there were no statistical differences; it seemed
248 that the experimental diets were well balanced and also had good palatability.
249 The hepatosomatix index (HSI) was similar in all treatments, similar to Nogales-Merida
250 et al. (2011a). On the contrary Piedecausa et al. (2007) reported a higher liver weight
251 when increasing theFO substitution level. Mesenteric fat was not affected by SO
252 inclusion, similar to results reported in the other fish oil substitution trials.
253 Whole body composition was similar in all treatments, showing that partial FO
254 substitution did not affect any parameter analysed. Moisture was the only parameter
255 that presented a statistical difference in Nogales-Mérida et al. (2011a).
256 Likewise, CPE and GEE were not affected by FO substitution, in agreement with the
257 results of Piedecausa et al. (2007) and Nogales-Merida et al. (2011a).
258 Our results prove the hypothesis established by Turchini et al. (2009) that the use of
259 vegetal oil does not significantly affect growth performance and feed utilisation in
260 omnivorous and herbivorous fish. However, with a few exceptions, most feeding trials
261 conducted to date have been relatively short-term. Long-term effects of dietary
262 inclusion of vegetal oil and animal fats remain to be elucidated, particularly with regard
263 to the effects on the fish immune system.
264 Numerous studies, have shown that fillet fatty acid composition is closely correlated to
265 dietary fatty acid composition and that feeding high levels of vegetable oils will
266 strongly influence the preferential deposition and retention of "unwanted" fatty acids
267 such as 18:2n-6 and 18:3n-3 in flesh lipids.
268 Fatty acids of muscle and liver of fish fed the experimental diets exhibited statistical
269 differences as a logical consequence of oil replacement. Piedecausa et al. (2007) and
270 Nogales-Merida et al. (2011a) also reported statistical differences with the total
271 replacement of FO in *D puntazzo*, concluding that despite its omnivorous habits,

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1
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3 272 sharpsnout sea bream presents a typical "marine" pattern with respect to fatty acid
4
5 273 deposition.
6
7 274 Despite the statistical differences obtained in EFA, such as LA, LNA, ARA, EPA and
8
9
10 275 DHA, sharpsnout sea bream seemed to have a certain capacity to elongate and de-
11
12 276 saturate from precursors (C18:2n-6 and C18:3n-3) to compensate the decrease of highly
13
14 277 unsaturated fatty acids (C20:4n-6, C20:5n-3 and C22:6n-3) in their diets (Fig. 3).
15
16 278 However, this compensation is not enough in fish fed the 75% FO diet. Martino et al.
17
18 279 (2002) tested different animal and vegetal oils in an omnivorous continental species,
19
20
21 280 *Pesudoplatystoma coruscans*, reporting a higher content of these EFA (special emphasis
22
23 281 on n-3 HUFA) in liver of fish fed soybean and linseed oil diets, justifying the results
24
25 282 with the fact that these species can bioconvert C_{18} PUFA to longer and more
26
27 283 unsaturated fatty acids (Turchini et al., 2009).
28
29
30 284 Soybean oil substitution had no apparent effect on sharpsnout sea bream liver
31
32 285 morphology. Hepatocytes exhibited a similar morphology in all treatments; it is likely
33
34 286 that no alteration was detected as fish liver is known to be diffuse and, in most cases, if
35
36 287 there is an alteration, it is usually focal (Roberts, 1981). The most common cause of
37
38
39 288 liver degeneration is fatty acid oxidation inducing fat infiltration in hepatocytes.
40
41 289 Caballero et al. (2002) reported homogeneous sized hepatocytes with some vacuolated
42
43 290 cytoplasm in trout fed with a 50% SO diet, which did not differ too much from the
44
45 291 control diet in relation to other vegetal oils. Figuereido-Silva et al. (2005) reported a
46
47
48 292 normal histological pattern in sea bass, despite the marked hepatocellular vacuolation,
49
50 293 corresponding to the naturally high lipid content, although hepatocytes displayed low
51
52 294 glycogen levels in sea bass fed a 50% SO diet. The lipid fraction of fish tissues is most
53
54 295 significantly affected by the nature of dietary lipids (vegetable oils versus fish oils),
55
56
57 296 triglycerides (neutral lipids) being much more affected than phospholipids (polar lipids).
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3 297 The livers of sharpsnout sea bream fed different SO substitution levels did not present
4
5 298 high amounts of lipid droplets in hepatocytes, possibly related to the temperature, just as
6
7 299 Ruyter et al. (2006) reported that trout fed at 12ºC presented a lowerlipid accumulation
8
9 300 in enterocytes than fish fed at 5ºC.
10
11 301 However, fish liver shows both an intra- and inter-specific variability. Such differences
12
13 302 from one species to another and from one individual to another could be correlated with
14
15 303 the amount of energy that they store (glycogen and/or lipid), depending on the specific
16
17 304 metabolic activities related to seasonal changes, temperature, sexual maturity and
18
19 305 nutritional status (Buslé and Gonzales i Anadon, 1996).
20
21 306 **Conclusion**
22
23 307 Sharpsnout sea bream juveniles can be fed with diets substituting fish oil by soybean oil
24
25 308 of up to 75% for a period of 84 days without affecting growth, feed efficiency
26
27 309 parameters biometric and body composition. Although the fatty acid profiles of the
28
29 310 liver and muscle reflect the diet profile, a marked reduction in HUFAs as in common
30
31 311 marine species was not observed, likely to be related to the capacity of this species to
32
33 312 bioconvert LA into EPA and DHA and LNA into ARA. This work also showed that the
34
35 313 liver morphology was not apparently affected by soybean oil inclusion in experimental
36
37 314 diets.
38
39 315 **Acknowledgements**
40
41 316 This research was supported by grants from the 'Planes Nacionales de Acuicultura
42
43 317 (JACUMAR)' in Spain.
44
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