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Effects of dietary different lipid sources on plasma metabolites in silvery-black porgy (*Sparidentex hasta*) juveniles

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Abstract

In the current study the plasma metabolites of silvery-black porgy (*Sparidentex hasta*) juveniles fed with different lipids sources were analyzed in order to bio-monitoring fish health condition. In this regard, an eight-week feeding trial was conducted using seven isonitrogenous (50%) and isolipidic (20%) experimental diets : FO, CO (canola oil), SO (sunflower oil), T (tallow), FC (fish oil + canola oil, 50:50), FS (fish oil + sunflower oil, 50:50) and FT (fish oil + tallow, 50:50). Fish fed with SO diet had the highest liver lipid content. Liver n−9 monounsaturated fatty acids, n−6 and n−3 poly unsaturated fatty acids were highest in fish fed the CO, SO and FO, respectively (p<0.05). Fish fed FO and T diets had the highest plasma total cholesterol, whereas fish fed CO and SO diets had the highest plasma triglyceride and VLDL levels (p<0.05). Plasma ALP and ALT were the highest in fish fed SO and FO groups, respectively. Fish fed FS diet had the highest plasma TBARS concentration, whereas plasma CAT activity was the highest in fish fed FO and FS diets. The results of this study demonstrated that different dietary lipid sources can drastically change fatty acid profile of the liver which consequently can alter different metabolic pathways that can affect plasma metabolite health indices in silvery-black porgy juveniles.

Keywords: Sparidae, Alternative lipid sources, Fatty acid profile, Lipid metabolism, Plasma metabolites

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Introduction

The progressive growth in global finfish aquaculture, static trend in the world capture fisheries and competition for using marine derivatives products (*i.e.* fish oil (FO) and fish meal (FM)) in the terrestrial animal feed industries have necessitated finding economically viable and environmentally friendly protein and lipid sources for the sustainability of the aquaculture industry (Tacon and Metian, 2008; Turchini *et al.,* 2009). In this regard, vegetable oils (VO) and terrestrial animal fats (TAF) have recognized as more sustainable alternatives to FO (Turchini *et al.,* 2009; 2010). However, these alternative lipid sources lack or have very limited content of essential fatty acids (EFA) especially n−3 long chain polyunsaturated fatty acids (n−3 LC-PUFA) that are characteristic of marine FO (Turchini *et al.,* 2010). These n−3 LC-PUFAs play important roles in neural development (Benítez-Santana *et al.,* 2012), growth (Skalli and Robin, 2004; Kim and Lee, 2004), endocrine function, ionic regulation (Glencross, 2009), immune responses (Zuo *et al.,* 2012) and reproduction (Izquierdo *et al.,* 2001). Thus, sparing limited FO resources by using alternative resources can raise challenges in aquafeed formulation. In this regards the plasma metabolites change as a consequence of different fatty acids profile delivered to the fish seems inevitable. On the other hand, the diversification of the aquaculture industry, which is based on social, economic and ecological considerations, is a main tool for the sustainability of this fast-growing industry. In this regard, silvery-black porgy (*Sparidentex hasta*, Valenciennes, 1830) considered as a potential candidate for aquaculture diversification in the Persian Gulf and Oman Sea regions (Pavlidis and Mylonas, 2011). In the recent years, this species has been regularly propagated in the Mariculture Research Station centers which are located in Khuzestan (South Iranian Aquaculture Research Center, Emam Khomeyni harbor, Iran) as well as Hormozgan (Kolahi, Minab, Iran) provinces and juvenile fish are transferred to sea cages for marine fish aquaculture extension projects (Mozanzadeh *et al.,* 2015a). Thus, this species has received a considerable attention from the scientific community in order to develop its intensive culture and improve diet formulation. Several studies in different fish species reported that different lipid sources as allosteric effectors on the activities of enzymes can affect metabolic pathways (*i.e.* glucogenesis and lipogenesis), which in turn may affect plasma metabolite indices in different fish species (Lee *et al.,* 2003; Peng *et al.,* 2008; Babalola *et al.,* 2009; Kenari *et al.,* 2011). In the current study, we analyzed plasma metabolites because they enable us to bio-monitoring the fish condition to determine the effects of alternative lipid sources on health condition of this species.

Material and methods

Experimental design

The efficiency of three alternative lipid sources, two vegetable oils, sunflower

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oil (SO) and canola oil (CO), terrestrial animal fat (tallow, T) and three blended lipids (FC: fish oil + canola oil, FS: fish oil + sunflower oil and FT: fish oil + tallow; 1:1) were evaluated and compared to a diet just containing FO (control diet). Seven isonitrogenous (*ca*. 50%) and isolipidic (*ca*. 20%) experimental diets were formulated (Tables 1 and 2). Fish oil was used in the control diet, which was substituted partially (50%) or totally (100%) with the alternative lipid sources in the other six diets. Fish meal, casein, gelatin and soybean meal were used as the main protein sources. Diets were prepared by mixing all ingredients for 30 min, after which, oil and sufficient distilled water were added to form soft dough, and then mechanically extruded to obtain pellets of the desired size (3 mm). The pellets were dried in a convection oven at 25 °C and stored in re-sealable plastic bags at −20 °C until use.

Table 1: Ingredients and proximate composition of the experimental diets (% dry diet).

	Diets*						
	FO	**CO**	**SO**	**T**	**FC**	**FS**	**FT**
Dietary ingredients [a]							
Fish meal [b]	40	40	40	40	40	40	40
Soybean meal [c]	18	18	18	18	18	18	18
Casein [d]	15	15	15	15	15	15	15
Gelatin	4	4	4	4	4	4	4
DL-Methionine [d]	1	1	1	1	1	1	1
L-lysine [d]	1	1	1	1	1	1	1
Raw corn starch	5	5	5	5	5	5	5
Fish oil [b]	10	–	–	–	5	5	5
Canola oil [e]	–	10	–	–	5	–	–
Sunflower oil [e]	–	–	10	–	–	5	–
Tallow[f]	–	–	–	10	–	–	5
Lecithin [e]	1	1	1	1	1	1	1
Antioxidant [g]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix [h]	2	2	2	2	2	2	2
Mineral premix [i]	2	2	2	2	2	2	2
Vitamin C[d]	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cr_2O_3[d]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)							
Dry matter	92.4	90.3	90.7	92.2	93.3	91.5	93.4
Crude protein	49.8	50.2	49.9	49.8	49.1	49.6	50.7
Crude lipid	19.1	20.2	20.4	20.8	20.6	20.3	20.7
Crude fiber	2.0	2.5	2.3	2.5	1.9	2.2	2.7
Crude carbohydrate	12.3	6.8	8.6	10.3	12.8	10.7	9.5
Ash	9.0	10.7	9.4	8.8	8.8	8.7	9.8

* Diet abbreviations are as follows: FO, fish oil; CO, canola oil; SO, sunflower oil; T, Tallow; FC, fish oil + canola oil; FS, fish oil + Sunflower oil; FT, fish oil + tallow.

[a] Proximate composition of ingredients as % Dry-weight basis [Fish meal (63.5% crude protein, 17.7% crude lipid); Casein (71.4% crude protein, 4.1%crude lipid); Gelatin (85% crude protein, crude lipid, 4.2); Soybean meal (41% crude protein, 4.2% crude lipid); Corn starch (2.4% crude protein, 3.7% crude lipid)].

[b] Parskilka, Mazandaran, Iran (*Clupeonella* sp.).

[c] Behpak indastrial company, Behshahr, Mazandaran, Iran.

[d] Sumchun, South Korea.

[e] Product of Kesht Va Sanat Shomal Vegetable oil Factories Complex, Neca, Mazandaran, Iran.

[f] Crude lamb tallow

[g] Butylated hydroxyl toluene, GarmabShimi, Iran.

[h] Vitamin premix U kg^{-1} of premix: vitamin A, 5000000 IU; vitamin D_3, 500000 IU; vitamin E, 3000 mg; vitamin K_3, 1500 mg; vitamin B_1, 6000 mg; vitamin B_2, 24000 mg; vitamin B_5, 52000 mg; vitamin B_6, 18000 mg; vitamin B_{12}, 60000 mg; Folic acid, 3000 mg; nicotinamide 180000 mg; antioxidant, 500mg, career up to1 kg, Damloran pharmaceutical company, Broujerd, Iran.

[i] Mineral premix mg kg^{-1} of premix: copper, 3000 mg; zinc, 15000 mg; manganese, 20000 mg; Iron, 10000 mg; potassium iodate, 300 mg, career up to 1 kg, Microvit®,Razak laboratories, Tehran, Iran.

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Table 2: Fatty acids composition of lipid sources and experimental diets (% of the total fatty acids).

Fatty acids	Fish oil	Canola oil	Sunflower oil	Tallow	Diets* FO	CO	SO	T	FC	FS	FT
14:0	2.3	0.1	0.1	3.5	3.0	1.3	1.5	3.9	2.1	1.7	3.2
16:0	15.9	4.3	6.5	23.5	19.9	13.4	12.9	21.1	15.3	15.5	21.9
18:0	4.2	1.8	3.8	0.2	4.9	3.8	4.9	12.6	4.0	5.1	9.5
SFA[1]	22.5	7.1	11.3	27.2	27.3	18.9	19.8	37.6	21.5	22.7	34.8
16:1n–7	4.3	0.2	0.1	1.1	4.2	1.4	0.3	2.1	2.8	3.0	3.2
18:1n–9	28.1	56.9	26.6	48.8	22.4	38.3	29.5	33.2	35.7	27.8	30.0
MUFA[2]	33.6	60.6	27.9	54.3	30.4	42.6	33.1	38.0	42.2	33.7	34.8
18:2n–6	2.3	23.0	58.5	0.9	7.5	19.3	24.3	6.3	12.7	21.2	6.1
18:3n–3	1.8	8.1	0.4	1.0	3.9	5.3	3.6	1.9	4.2	2.4	1.8
20:4n–6	0.6	0.0	0.0	0.0	0.4	0.3	0.3	0.2	0.4	0.3	0.4
20:5n–3	8.8	0.0	0.0	0.0	5.3	2.5	1.1	2.2	3.4	2.0	3.8
22:6n–3	26.2	0.0	0.0	0.0	16	7.4	7.8	7.0	11.4	9.9	11.6
PUFA[3]	43.5	31.7	59.2	3.9	34.6	38.1	39.5	18.3	33.5	37.9	25.5
n–3	40.1	8.1	0.4	1.0	27	15.2	12.5	11.1	19.0	14.3	17.2
n–6	3.3	23.6	58.8	2.9	7.9	19.6	24.6	6.5	13.1	21.5	6.5
n–9	26.4	60.4	26.6	48.8	23.7	41.1	31.8	34.7	39.2	30.6	31.5
n–3/n–6	12.2	0.3	0.0	0.3	3.4	0.8	0.5	1.7	1.5	0.7	2.6
ARA/EPA	0.07	0.0	0.0	0.0	0.1	0.1	0.3	0.1	0.1	0.2	0.1
DHA/EPA	3.0	0.0	0.0	0.0	3.0	3.0	7.1	3.2	3.4	5.0	3.1

* Diet abbreviations are as follows: FO, fish oil; CO, canola oil; SO, sunflower oil; T, Tallow; FC, fish oil + canola oil; FS, fish oil + Sunflower oil; FT, fish oil + tallow.
[1] SFA: saturated fatty acids also includes: 20:0 and 22:0.
[2]MUFA: monounsaturated fatty acids also includes: 14:1n-5, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9.
[3]PUFA: polyunsaturated fatty acids also includes: 18:4n-3, 20:2n-6 and 22:5n-3.

Fish maintenance and sampling

This study was carried out at the Mariculture Research Station of the South Iranian Aquaculture Research Center (SIARC), Emam Khomeyni harbor, Iran. Three hundred and thirty six juveniles of silvery-black porgy were randomly distributed into 21 cylindrical polyethylene tanks of 250 l volume, and each tank stocked with 16 fish [initial body weight 14.6±0.1 g., mean±standard deviation]. Fish were acclimated for 2 weeks before the onset of the nutritional trial. Tanks were supplied with filtered running seawater (1 l min^{-1}); salinity ranged between 47 and 49 ‰ (48.0±0.5 ‰) and temperature between 25 and 31 °C (28.9±1.5 ºC) during the experimental period (56 days). Average values for dissolved oxygen and pH were 6.8±0.4 mg L^{-1} and 7.7±0.2, respectively. The photoperiod was left under natural conditions (30°32′N, 49°20′E; 14 L: 10 D). Diets were tested by triplicate; fish were fed by hand to visual satiation three times per day (0900h, 1300h and 1700h) for 56 days. At the end of the trial, fish were fasted for 24 h before being anaesthetized (2-phenoxyethanol at 0.5 ml L^{-1}; Merck, Schuchardt, Germany). At the end of the experiment three specimens from each replicate were anaesthetized with 2-phenoxyethanol and then euthanized with an overdose of this anaesthetic to evaluate the FA profile of their liver. Blood was collected from the caudal vein in 4 fish (n=12 fish per diet treatment, n = 4 fish per replicate) with heparinized syringes, and centrifuged (4000 g, 10 min, 4 ºC) and plasma separated and stored at -80 °C until their analysis.

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Fatty acids analysis

Total lipids from the liver and diets were extracted by sample homogenization in chloroform/methanol (2:1, v v^{-1}) according to method of Folch *et al.*, (1957). Methyl esters were prepared by transmethylation using methanolic KOH and n-heptane according to ISO5509 method (1978) with minor modification (Agh *et al.*, 2014). The FA composition of liver and diets were determined by an auto sampler gas chromatography (GC, Agilent technologies 7890N, USA), equipped with a flame ionization detector (FID) and a cyanopropyl-phenyl capillary column (DB-225MS, 30 m×0.250 mm ID×0.25 µm Film thickness, USA). Identification of the FA was performed by comparing their retention time with those of an external commercial standard mixture (GLC-68d, Nu-Chek Prep., MN, USA) according to Agh *et al.* (2014).

Plasma metabolites analyses

Plasma metabolites were analyzed by means of an auto-analyzer (Mindray BS-200, China) using commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were conducted for glucose, total protein, albumin, total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), alkaline phosphatase (ALP) and alanine aminotransferase (ALT). Moreover, the content of total globulin was estimated by subtracting albumin from total protein (Kumar *et al.*, 2005). Lipid peroxidation levels in plasma were estimated from the production of malondialdehyde (MDA) determined using the TBARS assay, which measures the levels of thiobarbituric acid reactive substances (Buege and Aust, 1978). The activity of catalase (CAT) was determined using the method described by Koroluk *et al.*, (1988).

Statistical analyses

Data were analyzed by using SPSS ver.15.0 (Chicago, Illinois, USA). All the data are presented as mean±standard error of the mean calculated from three biological replicates. Arcsine transformations were conducted on all data expressed as percentages. One way ANOVA was performed at a significance level of 0.05 following confirmation of normality and homogeneity of variance. Duncan's procedure was used for multiple comparisons when statistical differences were found among groups by the one-way ANOVA.

Results

Liver FA profile

In the present study we evaluate lipid content and FA profile of the liver which is the main organ in lipid metabolism and FA synthesis. Fish fed SO diet had the highest liver lipid content; however, fish fed FO, CO and FT diets had the lowest lipid percentage in the liver and the other groups showed the intermediate values (Table 3) (p<0.05). Total saturated fatty acids (mainly 16:0 and 18:0) and

[DOR: 20.1001.1.15622916.2020.19.1.14.6]

monounsaturated fatty acids (primarily oleic acid; OA; 18:1n–9) had the highest level in fish fed T and CO diets, respectively (p<0.05; Table 3). The concentrations of total n−6 PUFA (especially linoleic acid; LA; 18:2n–6) and n–3 LC-PUFA (mainly docosahexaenoic acid; DHA; 22:6n–3) had the highest level in fish fed SO and FO diets, respectively. The levels of arachidonic acid (ARA; 20:4n–6) was lower in fish fed FC and FS diets than the other groups (p<0.05). The concentration of ALA in the liver of fish fed CO, SO and FS diets was higher than other groups (p<0.05). The levels of eicosapentaenoic acid (EPA; 20:5n–3) was highest in fish fed FO and FC diets and lowest in fish fed CO diet (p<0.05).

Table 3: Lipid content (%) and fatty acids profile of liver (% of the total fatty acids) of *Sparidentex hasta* juveniles fed different experimental diets (means±SEM, n=3). A different superscript in the same row denotes statistically significant differences (p<0.05).

	Diets*						
Fatty acids	**FO**	**CO**	**SO**	**T**	**FC**	**FS**	**FT**
Lipid content	21.7 ± 1.5^{b}	22.0 ± 2.8^{b}	28.3 ± 1.2^{a}	24.0 ± 1.3^{ab}	26.1 ± 2.3^{ab}	24.9 ± 0.9^{ab}	23.1 ± 1.7^{b}
14:0	1.6 ± 0.2^{abc}	1.3 ± 0.2^{c}	1.5 ± 0.1^{bc}	2.1 ± 0.1^{ab}	1.2 ± 0.1^{c}	2.3 ± 0.2^{a}	1.9 ± 0.1^{abc}
16:0	15.6 ± 0.5^{a}	9.1 ± 0.3^{c}	18.0 ± 0.5^{a}	19.5 ± 0.8^{a}	12.6 ± 0.9^{b}	19.1 ± 0.5^{a}	17.9 ± 0.5^{a}
18:0	9.4 ± 0.3^{bc}	3.6 ± 0.1^{e}	7.8 ± 0.5^{cd}	12.4 ± 0.3^{a}	2.3 ± 0.2^{e}	6.4 ± 0.2^{d}	9.9 ± 0.5^{b}
SFA[1]	26.7 ± 0.9^{ab}	15.4 ± 0.1^{c}	27.9 ± 1.3^{b}	36.2 ± 1.5^{a}	16.6 ± 0.3^{cd}	29.6 ± 3.1^{bc}	29.9 ± 1.2^{ab}
16:1n–7	2.5 ± 0.1^{bc}	2.8 ± 0.2^{ab}	1.3 ± 0.2^{c}	2.4 ± 0.2^{bc}	2.2 ± 0.3^{bc}	3.8 ± 0.4^{a}	2.3 ± 0.2^{bc}
18:1n–9	24.8 ± 0.8^{cd}	48.3 ± 1.4^{a}	29.0 ± 0.8^{cd}	31.0 ± 1.3^{bc}	36.0 ± 2.5^{b}	22.6 ± 1.5^{d}	29.9 ± 0.9^{bc}
MUFA[2]	36.7 ± 0.8^{b}	52.2 ± 1.8^{a}	31.7 ± 1.2^{bc}	34.8 ± 1.6^{bc}	43.8 ± 2.8^{b}	27.1 ± 1.9^{d}	33.0 ± 1.0^{bc}
18:2n–6	6.0 ± 0.3^{c}	9.9 ± 0.2^{b}	20.2 ± 0.5^{a}	3.8 ± 0.3^{d}	6.7 ± 0.2^{c}	11.2 ± 0.3^{b}	5.6 ± 0.3^{c}
18:3n–3	1.8 ± 0.3^{c}	5.8 ± 0.6^{a}	5.5 ± 0.3^{a}	1.0 ± 0.6^{c}	3.9 ± 0.2^{b}	5.6 ± 0.2^{a}	1.0 ± 0.2^{c}
20:4n–6	1.0 ± 0.3^{a}	0.5 ± 0.2^{ab}	0.9 ± 0.1^{ab}	1.0 ± 0.2^{a}	0.2 ± 0.1^{b}	0.2 ± 0.0^{b}	0.5 ± 0.1^{ab}
20:5n–3	3.0 ± 0.1^{a}	1.2 ± 0.2^{b}	1.5 ± 0.1^{ab}	1.7 ± 0.2^{ab}	3.0 ± 0.2^{a}	2.5 ± 0.5^{ab}	2.1 ± 0.3^{ab}
22:6n–3	16.3 ± 0.7^{a}	8.7 ± 0.4^{b}	9.0 ± 0.3^{b}	8.2 ± 0.4^{b}	15.0 ± 1.1^{a}	14.1 ± 0.8^{a}	13.8 ± 0.3^{a}
PUFA[3]	32.4 ± 1.4^{abc}	26.8 ± 1.5^{cd}	38.6 ± 2.0^{a}	17.2 ± 1.4^{e}	29.7 ± 1.6^{bcd}	35.4 ± 2.1^{ab}	24.5 ± 1.1^{de}
n–3	24.1 ± 1.0^{a}	15.7 ± 1.1^{cd}	16.1 ± 0.8^{c}	10.9 ± 0.6^{d}	21.8 ± 1.2^{ab}	22.2 ± 1.5^{ab}	17.0 ± 0.8^{bc}
n–6	8.3 ± 0.8^{cd}	11.1 ± 0.5^{bc}	22.6 ± 1.3^{a}	6.2 ± 0.8^{d}	7.9 ± 0.4^{cd}	13.2 ± 0.6^{b}	7.5 ± 0.3^{cd}
n–9	29.3 ± 0.6^{b}	49.1 ± 1.5^{a}	29.9 ± 1.0^{b}	31.8 ± 1.4^{b}	36.4 ± 2.5^{b}	22.8 ± 1.5^{c}	30.5 ± 0.9^{b}
n–3/n–6	2.9 ± 0.2^{a}	1.4 ± 0.1^{d}	0.7 ± 0.1^{e}	1.8 ± 0.1^{cd}	2.8 ± 0.1^{ab}	1.7 ± 0.1^{cd}	2.3 ± 0.1^{bc}
ARA/EPA	0.4 ± 0.2^{ab}	0.4 ± 0.1^{ab}	0.6 ± 0.0^{a}	0.6 ± 0.1^{a}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.3 ± 0.0^{b}
DHA/EPA	5.7 ± 0.7	7.1 ± 0.6	5.9 ± 0.4	4.8 ± 0.3	5.0 ± 0.0	5.7 ± 0.7	6.6 ± 0.8

* Diet abbreviations are as follows: FO, fish oil; CO, canola oil; SO, sunflower oil; T, Tallow; FC, fish oil + canola oil; FS, fish oil + Sunflower oil; FT, fish oil + tallow.
[1] SFA: saturated fatty acids also includes: 20:0 and 22:0.
[2]MUFA: monounsaturated fatty acids also includes: 14:1n-5, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9.
[3]PUFA: polyunsaturated fatty acids also includes: 18:4n-3, 20:2n-6 and 22:5n-3.

Plasma metabolites

The plasma metabolites of fish were significantly affected by different lipid sources (Table 4). Plasma glucose was the highest in fish fed SO diet; however, fish fed FO and FT diets demonstrated the lowest plasma glucose levels and other groups showed intermediate values (p<0.05). Plasma total cholesterol was the highest in fish fed FO and T diets, but the lowest in fish fed FC diet. Fish fed CO and SO diets had the highest plasma triglyceride and VLDL levels, whereas

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fish fed FO, FC and FT showed the lowest values for these parameters (p<0.05). On the other hand, fish fed FO and FT diets had the highest and lowest plasma HDL levels, respectively. Fish fed SO, T and FC diets had the highest plasma LDL levels and fish fed CO and FS diets had the lowest values for plasma LDL (p<0.05). Plasma ALP and ALT were the highest in fish fed SO and FO groups, respectively (p<0.05). Fish fed FS and FT diets had the highest and lowest plasma TBARS concentrations, respectively (p<0.05). Plasma CAT activity was the highest in fish fed FO and FS diets, whereas the lowest in fish fed T and FT diets (p<0.05). Plasma total protein, albumin and globulin were stable among different dietary treatments (p>0.05).

Table 4: Plasma biochemical profile of *Sparidentex hasta* juveniles fed different experimental diets (means±SEM, n=3). A different superscript in the same row denotes statistically significant differences (p<0.05).

	Diets*						
	FO	**CO**	**SO**	**T**	**FC**	**FS**	**FT**
Glucose (g dl^{-1})	109.0 ± 7.6^{d}	133.3 ± 2.0^{bc}	154.0 ± 6.5^{a}	125.3 ± 4.8^{bc}	120.0 ± 5.3^{cd}	136.3 ± 4.1^{b}	106.7 ± 1.5^{d}
Protein components							
Total Protein (g dl^{-1})	4.0 ± 0.4	4.2 ± 0.3	3.7 ± 0.3	3.7 ± 0.2	3.8 ± 0.2	3.3 ± 0.1	3.2 ± 0.2
Albumin (g dl^{-1})	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0
Globulin (g dl^{-1})	3.5 ± 0.3	3.7 ± 0.4	3.2 ± 0.2	3.2 ± 0.1	3.3 ± 0.2	3.0 ± 0.1	2.9 ± 0.2
Lipid components							
Total cholesterol (mg dl^{-1})	246.7 ± 56.8^{a}	213.0 ± 19.6^{ab}	188.3 ± 11.0^{abc}	231.0 ± 13.4^{a}	127.3 ± 19.8^{c}	157.3 ± 18.4^{bc}	201.0 ± 21.7^{ab}
Triglyceride (mg dl^{-1})	210.3 ± 13.8^{c}	292.3 ± 19.3^{a}	302.3 ± 24.6^{a}	241.0 ± 8.9^{bc}	210.3 ± 22.2^{c}	275.0 ± 10.4^{ab}	186.3 ± 10.1^{c}
HDL (mg dl^{-1})[1]	109.7 ± 9.0^{a}	70.7 ± 11.6^{bcd}	93.3 ± 6.5^{ab}	55.0 ± 3.6^{dc}	87.0 ± 7.2^{ab}	80.0 ± 10.1^{bc}	51.0 ± 4.4^{d}
LDL (mg dl^{-1})[2]	39.0 ± 4.0^{ab}	34.0 ± 1.2^{b}	47.0 ± 0.6^{a}	48.3 ± 1.5^{a}	49.7 ± 8.4^{a}	32.0 ± 3.5^{b}	37.0 ± 2.3^{ab}
VLDL (mg dl^{-1})[3]	42.1 ± 5.1^{c}	58.5 ± 1.2^{a}	60.5 ± 3.5^{a}	48.2 ± 1.5^{bc}	42.1 ± 8.4^{c}	55.0 ± 0.6^{ab}	37.3 ± 2.3^{c}
Enzymes							
ALP (U l^{-1})[4]	886.7 ± 86.0^{b}	758.3 ± 54.3^{bc}	1228.7 ± 63.8^{a}	631.3 ± 51.3^{bc}	566.0 ± 78.5^{c}	870.3 ± 1.5^{b}	647.0 ± 54.8^{bc}
ALT(U l^{-1})[5]	907.0 ± 217.0^{a}	609.7 ± 177.1^{ab}	362.0 ± 162.8^{b}	577.8 ± 134.7^{ab}	376.3 ± 179.8^{b}	230.0 ± 60.8^{b}	208.0 ± 60.8^{b}
Oxidative status							
TBARS (µmol MDA L^{-1})	78.1 ± 4.7^{c}	53.8 ±7.4^{d}	101.0 ± 4.8^{ab}	74.9 ± 13.7^{cd}	100.0 ± 5.2^{ab}	107.5 ± 2.5^{a}	16.8 ± 8.3^{e}
Catalase activity (µmol min^{-1} ml^{-1})	0.23 ± 0.0^{a}	0.15 ± 0.0^{abc}	0.20 ± 0.0^{ab}	0.03 ± 0.0^{c}	0.14 ± 0.0^{abc}	0.22 ± 0.0^{a}	0.08 ± 0.0^{c}

* Diet abbreviations are as follows: FO, fish oil; CO, canola oil; SO, sunflower oil; T, Tallow; FC, fish oil + canola oil; FS, fish oil + Sunflower oil; FT, fish oil + tallow. [1]HDL: high density lipoprotein, [2]LDL: low density lipoprotein, [3]VLDL: very low density lipoprotein, [4]ALP: alkaline phosphatase, [5]ALT; alanine aminotransferase.

Discussion

Liver FA profile

In carnivorous marine fish species with limited desaturase–elongase ability to produce LC-PUFA from their precursors primarily ALA and LA, diet is the main determinant of body FA profile (Tocher, 2010). In the present study fish fed SO diet had the highest liver lipid content, it may be due to an increase in LA level in the liver and the imbalance in the n−3/n−6 ratio, as well as changes in lipid metabolism by reducing the activity of enzymes involved in the esterification of free fatty acids (FFA) into triacylglycerol and phospholipid for VLDL production (Ruyter *et al.*, 2006). This phenomenon

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leads to increasing lipid deposition in the liver and might result in liver steatosis as also have been reported in other fish species (Caballero *et al.*, 2004; Ruyter *et al.*, 2006). The concentration of SFA was the highest in the liver of fish fed T diet may probably due to higher levels of SFA in TAF sources (Turchini *et al.*, 2010). On the other hand, fish fed CO diet had the lowest levels of SFA in the liver as also reported in other marine fish species fed CO as an alternative lipid source in their diets (Turchini *et al.*, 2010; Nasopoulou and Zabetakis, 2012). Moreover, concentration of total MUFA especially OA were the highest in the liver of fish fed CO diet which is in line with the results reported for other marine fish species (Sales and Glencross, 2011; Turchini *et al.*, 2010; Bowyer *et al.*, 2012). We detected higher concentration of total n–6 PUFA especially LA; but lower n–3 / n–6 ratio in the liver of fish fed SO and FS diets. Similar results also reported in other marine fish species fed SO as an alternative lipid source in their diets (Lin *et al.*, 2007; Wassef *et al.*, 2007; Wijekoon *et al.*, 2014). The concentration of ARA in fish liver was equal to the level of this FA in respective diets, which might indicate negligible using high levels of LA in VO sources as a consequence trifling activity of Δ 6-desaturase in silvery-black porgy. The DHA content of liver was relatively higher than concentration of this FA in respective diets, indicating selective retention of DHA in liver tissue as also has been reported in the other studies in the same species (Mozanzadeh *et al.*, 2015b; 2016a; 2016b). The selective deposition of DHA in certain tissues such as liver might be due to the importance of this n–3 LC-PUFA as a major structural component of cell membrane, relative resistance to β-oxidation and high specificity of fatty acyl transferases for it (Bell *et al.*, 2001).

Plasma metabolites

In the present study, fish fed SO diet had the highest plasma glucose. The increase of the plasma glucose in fish fed SO diet may be as a result of impairment of glucose mobilization or increment of plasma FFA in this group which associated with the high liver lipid content in fish fed this diet. It is suggested that, high plasma FFA concentration led to glucose increasing via increased expression of hepatic glucose-6-phosphatase (Massillon *et al.*, 1997). In this context, Luo *et al.*, (2014) reported that dietary coconut oil led to an increase non-esterified fatty acids and glucose in plasma in comparison with fish fed dietary FO in rainbow trout (*Oncorhynchus mykiss*). On the other hand, the pentose phosphate pathway enzymes (*i.e.* glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP-isocitrate dehydrogenase) activities has been found to be higher in the liver of Atlantic salmon fed diets contained VO sources compared to fish fed FO diet which led to an increase in plasma glucose concentration (Jordal *et al.*, 2007; Sissener *et al.*, 2013). Similar results also reported that dietary VO sources led to an increase in plasma

[DOR: 20.1001.1.15622916.2020.19.1.14.6]

glucose in planet catfish (*Heterobranchus longifilis*; Babalola *et al.,* 2009) and Caspian brown trout (*Salmo trutta caspius;* Kenari *et al.,* 2011). The stable plasma protein components showed in this study indicating consistent homeostasis condition in fish fed different lipid source as also reported in other fish species fed with different alternative lipid sources (Lee *et al.,* 2003; Peng *et al.,* 2008; Babalola *et al.,* 2009; Kenari *et al.,* 2011). In the present study, plasma cholesterol in fish fed FO and T diets was relatively higher than those in the other experimental groups, and may in order to high level of total SFA and cholesterol in animal fats. In fact SFA decrease LDL receptors activity by decreasing cell membrane fluidity, which affect LDL-cholesterol biding to the receptors, decreasing LDL fractional catabolic rate, decreasing LDL receptors gene expression and finally increasing LDL apolipoprotein B receptor activity that leading to an increase in plasma cholesterol (Fernandez and West, 2005). Similar results also reported in other fish species fed diets terrestrial animal fat sources such as starry flounder (*Platichthys stellatus*; Lee *et al.,* 2003) black seabream (*Acanthopagrus schlegeli;* Peng *et al.,* 2008) and rainbow trout (Luo *et al.,* 2014). Furthermore, higher plasma triglyceride and VLDL levels in fish fed VO diets in this study may indicate higher liver FA synthesis in these groups. It is suggested that, VO stimulates liver triglyceride production and secretion as a result of high levels of OA and LA in these lipid sources (Vegusdal *et al.,* 2005; Ruyter *et al.,* 2006; Kjær *et al.,* 2008). In this context, Caballero *et al.,* (2006) reported that lipogenesis increased in the liver of gilthead seabream (*Sparus aurata*) fed diets containing soybean and rapeseed oils. The above mentioned authors speculated that LA enhances phosphatidylcholine synthesis which is responsible for the increase in lipid and VLDL secretion through glycerol-3-phosphate pathway; however, OA reduces the re-acylation activity of the lipids in both of the re-esterification pathways (glycerol-3-phosphate and the monoacylglycerol). On the other hands, in the present study fish fed SO and CO diets had lower n–3 / n–6 ratio in the liver than fish fed the other diets, which may be induced liver lipid secretion in these groups. In this context Kjær *et al.* (2008) reported that low n–3 / n–6 ratio in endogenous FA composition of the hepatocytes induce hepatic triglycerides-rich VLDL particle secretion rate. Similarly, Kenari *et al.* (2011), Liland *et al.* (2013) and Luo *et al.* (2014) also reported that dietary VO led to an increase in plasma triglyceride in Caspian brown trout, Atlantic salmon and rainbow trout, respectively. Fish fed FO diet had the highest plasma HDL levels, may because of high levels of n–3 LC-PUFA in this diet. It is believed that, the mechanism whereby n–3 LC-PUFA increase HDL levels may be related to the down-regulation of the cholesteryl ester transfer protein and lecithin-cholesterol acyltransferase enzymes (Abbey *et al.,* 1990). In this regard, Mozanzadeh *et al.* (2015b;

[DOR: 20.1001.1.15622916.2020.19.1.14.6]

2016a) also reported that plasma HDL levels increased as dietary n–3 LC-PUFA increased in silvery-black porgy. Alkaline phosphatase is one of the most reliable hepatic biomarkers for assessing nutritional and health condition in silvery-black porgy (Mozanzadeh *et al.,* 2015c). In the current study, fish fed SO diet had the highest ALP levels may be due to lipid accumulation in the liver so that may cause hepatocytes steatosis and injury. Similarly, Caballero *et al.,* (2004) reported that replacement of dietary FO with soybean oil induced lipid accumulation in the liver and hepatocyte steatosis in gilthead seabream. Moreover, fish fed FO diet had the highest plasma ALT, may as a result of an increase in liver n–3 LC-PUFA levels and lipid peroxidation. The results of the present study showed that the levels of plasma TBARS was the highest in fish fed with SF diet may be because of elevated concentrations of PUFA (especially LA) and n–3 LC-PUFA (mainly DHA) in this diet which can lead to the production of reactive oxygen species and cause the oxidation of membrane-bound fatty acids (Rueda-Jasso *et al.,* 2004; Mozanzadeh *et al.,* 2015b; 2016a). On the other hand, plasma CAT activity increased with increasing TBARS values, indicating sign of oxidative stress in fish fed diet with high LC-PUFAs levels. Similar results also reported in other fish species fed high LC-PUFA content diets (Mozanzadeh *et al.,* 2015b; 2016a). In conclusion, the results of this study demonstrated that different dietary lipid sources can profoundly affect plasma metabolites as a consequence of drastic changes in FA profile of liver.

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