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Fatty Acid Composition of Fry Mirror Carp (*Cyprinus carpio*) Fed Graded Levels of Sand Smelt (*Atherina boyeri*) Meal*

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ABSTRACT : The effect of replacement of fish meal (FM) in diets with sand smelt meal (SSM) on fatty acid composition of carp fry, *Cyprinus carpio*, was examined. Five isonitrogenous and isoenergetic (38% crude protein, 15.75 kJ g^{-1}) diets replacing 0, 25, 50, 75, and 100% FM protein by SSM protein were formulated. Each diet was randomly allocated to triplicate groups of fish in aquaria, and each aquarium was stocked with 20 fish (initial average weight of 0.300±0.65 g $fish^{-1}$). Fish were fed twice daily to apparent satiation for 13 weeks. Results indicated that final weight, specific growth rate and feed efficiency ratio of fish fed with different SSM replacement diets did not differ significantly ($p>0.05$) from fish fed the control diet, except for 100% SSM level. No significant differences were noted among experimental treatments on dry matter, protein, lipid and ash contents of the fish body composition ($p>0.05$). Fatty acid analysis showed that saturated fatty acids in fish muscle significantly decreased, but monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) did not change with increasing dietary SSM. However, some changes also could be observed for some particular fatty acids in experimental fish. For example, the amounts of 15:0, 17:0, 18:1n-7, 18:2n-6 and 22:5n-3 significantly increased, but 16:0, 18:1n-9, 18:3n-3 and 20:1 n-9 significantly decreased with increasing dietary SSM. Total n-6 PUFA increased with increasing dietary SSM, but total n-3 PUFA were not changed in muscle of fish fed the experimental diets. The ratio of n-3 to n-6 was not affected significantly in muscle of fish fed the experimental diets containing different proportions of SSM, including the control diet. (**Key Words :** *Cyprinus carpio*, Fishmeal Substitute, Sand Smelt Meal, Fatty Acids)

INTRODUCTION

Researchers have recently intensified interest in the relationships between the fatty acid composition of fish and that of the diet (Tocher et al., 2004; Ng et al., 2006; Bahurmiz and Ng, 2007; Gümüş and Erdogan, 2010). The lipid content of diet is important as a source of dietary energy, but is also fundamental for supplying adequate amount of essential fatty acids (EFA) (Sargent et al., 2002).

Fish meal (FM) is an excellent source of essential amino acids. FM from oily fish species may contain up to 8-10% of residual fat, which commonly contains from 20 to 35% highly unsaturated n-3 fatty acids. Hence, if FM is included in the diet as low as 30 g/100 g it will provide from 0.5 to 1% of the dry diet highly unsaturated n-3 fatty acids, and the general EFA requirements of most commonly farmed finfish species will be fulfilled regardless of the dietary lipid source included in the formulation. If residual fish oil is fully substituted with a complete or partial replacement of the fish meal fraction of the diet, the risk of a net deficiency in EFA is possible, and an appropriate source of EFA needs to be included in the diet formulation (Sargent et al., 2002).

The efficiencies of the various alternative animal protein sources as replacements for fish meal have been evaluated in fish diets, e.g. poultry by-product meal (Yang et al., 2006), gambusia meal (Ahmad, 2008), tuna liver meal (Gümüş et al., 2009) and sand smelt meal (Gümüş et al., 2010). Although the usage of alternative terrestrial or aquatic protein sources as replacement of fish meal in fish diets is expanding (Hardy, 2006), there are concerns about the adverse effects at extensive replacement rates on the lipid composition in aquaculture diets (Pigott, 1989; Ahlgren et al., 1994). Thus, ensuring adequate enrichment with fish oil or other sources of EFA by means of EFA requirements and sufficient fatty acids retainment in the muscle tissue is needed.

Gümüş et al. (2010) stated that sand smelt meal (SSM), among other sources of animal origin, is a rich protein

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source. They also reported adequate growth increase in the Nile tilapia fry, fed with diets replaced by SSM for FM. Similarly, when used as replacement of FM as protein source has supplied for mirror carp fry, it is unknown how replacement rates of SSM would affect the fatty acid composition of fish muscle, and consequently their fat quality. The objective of this study was to analyse fatty acid changes of mirror carp fry when fish meal is gradually replaced by SSM as an alternative protein source.

MATERIALS AND METHODS

Experimental diets

Sand smelt meal (SSM) was from the same source and prepared as in the Nile tilapia fry study (Gümüş et al., 2010). Five isonitrogenous (38% crude protein) and isoenergetic (15.75 kJ DE g^{-1}) experimental diets replacing 0 (the control group), 25, 50, 75 and 100% of FM protein by SSM protein were formulated (Table 1). All ingredients were ground to a fine powder (particle size 0.550-mm) using an experimental hammer mill and were thoroughly mixed in a food mixer and the mixture was then pelleted with meat grinder through a 2-mm die and dried for about 24 h in a ventilated oven at 70°C. Before feeding, the dried diets were crushed and sieved into suitable particle sizes (0.8-2 mm diameter) for the fry and stored at -20°C in airtight polyethylene bags, until required.

Experimental conditions and feeding

Experimental fish, obtained from a local fish hatchery (Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey), were acclimated to laboratory conditions for 2 weeks in a 250-L glass aquarium prior to the start of the experiment. The experiment was carried out between 15 July and 15 October of 2008 at the Laboratory of Fisheries, Faculty of Akdeniz University, Antalya, Turkey. At the start of the experiment, to maintain three replicates per treatment, randomly selected 15 groups of twenty fry (0.300±0.65 g $fish^{-1}$) were stocked into separate 65-L glass aquaria. Fish were fed to apparent satiation twice daily (09:00 and 16:30 h) for 13 weeks. The aquaria were filled with dechlorinated tap water throughout the study. Continuous aeration for each aquarium was provided using a blower and stones.

During the experimental period, the water temperature ranged from 24 to 26°C, dissolved oxygen from 4.8 to 5.5 mg/L, pH from 7.8 to 8.4. Water quality parameters remained within the acceptable ranges for carp fry growth during the course of the study (Horvath et al., 2002). The experimental period a diurnal light/dark cycle of 12:12 h was maintained during the feeding trials.

Analytical methods

A sample of 40 fish from stock fish at the initiation of feeding experiment and 15 random fish per aquarium at the termination were collected and stored frozen (-20°C) for determination of proximate and fatty acid composition. Proximate composition was conducted by standard methods of AOAC (1995). Samples of main protein ingredients, diets and fish muscle were dried to a constant weight at 105°C to determine moisture. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl metod; lipid by ether extraction using Soxhlet; ash by combustion at 550°C, fibre by acid and alkaline extraction using a

Table 1. Feed formulation of the experimental diets

Ingredients (%)	Graded levels of SSM in diets (%)				
	0 (control)	25	50	75	100
Fish meal	44.99	33.74	22.49	11.25	0
Sand smelt meal	0	10.54	21.07	31.6	42.13
Soybean meal	16.85	16.85	16.85	16.85	16.85
Corn starch	23.05	23.05	23.05	23.05	23.05
Soybean oil	3.0	3.0	3.0	3.0	3.0
Fish oil	1.9	1.7	1.47	1.25	1.05
Vitamin premix[1]	2.0	2.0	2.0	2.0	2.0
Mineral premix[2]	3.0	3.0	3.0	3.0	3.0
L-methionine	0.2	0.2	0.2	0.2	0.2
Iodized salt	0.1	0.1	0.1	0.1	0.1
$CaHPO_4 2H_2O$	2.0	2.45	2.85	3.38	3.8
CMC[3]	1.0	1.0	1.0	1.0	1.0
Cellulose	1.41	1.87	2.42	2.82	3.32
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

[1,2] Gümüş et al. (2010). [3] Carboxymethyl cellulose.

Whelp model extractor and nitrogen free extract (NFE) by subtracting the other components from 100.

Lipid contents of main protein ingredients, experimental diets and fish muscle were determined by Soxhlet extraction using *n*-hexane, and then fatty acid contents were determined by preparation of methyl esters as described by IUPAC (1979). Methylated samples were performed on a Perkin Elmer AutoSystem XL Gas Chromatography equipped with a 30 m×0.25 mm×0.20 μm ID fused silica capillary column. The flame ionization detector (FID) and injector port were maintained at 260 and 240°C, respectively. Column heating was performed, starting from 120°C and increasing to 220°C at 5°C per minute. A quantity of 1 μl of the samples was injected on the column, at a split rate of 1:50. The flow rate of helium carrier gas was 0.5 ml/min, hydrogen 45 ml/min, and air 450 ml/min. Free fatty acids were identified by comparison of retention time of the gas chromatographic peaks with those of commercial free fatty acid methyl ester standards. They were automatically computed as a percentage by the data processor (Chrom-card) from the ratio of individual peak area to the total peaks area of fatty acids.

Growth parameters

Growth performance and feed utilization were measured in terms of weight gain per day (WG, %), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), and survival (%). These parameters were calculated as follows: Survival (%) = Final fish number/initial fish number×100; WG (%) = ((FW(g)-IW(g))/IW(g))×100; SGR (% day^{-1}) = ((Ln FW(g)-Ln IW(g))/time days×100); FCR = FI (g)/(FW(g)-IW(g)); where FI, IW and FW are feed intake, initial and final weight, respectively.

Statistical analysis

All data were subject to one-way analysis of variance (ANOVA) using SPSS 15.0 for Windows. Differences between the means were tested by Duncan's multiple range tests. The level of significance was chosen at $p>0.05$ and the results are presented as mean±SD (Steel et al., 1996).

RESULTS

Ingredient composition

The proximate and fatty acid composition in main protein ingredients SSM, fish meal (FM) and soybean meal (SBM) are shown in Table 2. The crude ash content of SSM (9.83%) was lower than that of FM (15.41%), but slightly higher as compared to SBM (6.86). However, the levels of crude protein and total lipid (71.19% and 9.83%, respectively) were higher than both FM (66.71% and 6.68%) and SBM (46.17% and 1.08%). Thus, according to these parameters, the chemical composition quality of SSM was found to be similar with FM but higher than SBM. Concerning the fatty acid composition of the ingredients, MUFA content was the highest in SSM (29.43%), but SFA was moderate in comparison with FM (39.45%). On the other hand, amount of PUFA (28.18%) in SSM was distinctly lower than that of FM containing considerable amounts of PUFA (39.19%) mainly composed of eicosapentaenoic acid (EPA, 20:5n-3) (16.02%) and docosahexaenoic acid (DHA, 22:6n-3) (18.52%). These two acids, to be found at lower amounts in SSM, were absent in SBM. SSM contained large amount of n-6, but n-3 values were lower than those of FM.

Table 2. The total fatty acids (%, on total fatty acids) and proximate composition (%, on wet wt.) of sand smelt meal (SSM), fish meal (FM) and soybean meal (SBM)

Parameters	SSM	FM	SBM
Crude protein	71.19	66.71	46.17
Crude lipid	9.18	6.68	1.08
Crude fibre	0.40	0.40	3.90
Dry matter	91.11	89.42	86.84
Ash	9.83	15.41	6.86
NFE[1]	0.50	0.20	28.82
Fatty acids[2]			
14:0	3.75	4.48	1.53
15:0	1.73	0.35	-
16:0	24.79	27.48	23.14
17:0	1.7	0.58	-
18:0	7.48	7.28	7.12
16:1n-7	10.23	4.41	1.04
18:1n-7	8.58	4.86	2.56
18:1n-9	10.62	9.28	16.04
18:2n-6	3.53	0.91	40.14
18:3n-6	2.32	0.4	5.41
18:3n-3	0.70	1.05	0.77
20:4n-6	3.73	1.34	-
20:5n-3	7.99	16.02	-
22:5n-3	3.1	0.95	-
22:6n-3	6.81	18.52	-
SFA	39.45	40.17	32.2
MUFA	29.43	18.55	19.64
PUFA	28.18	39.19	46.32
n-3 PUFA	18.60	36.54	0.77
n-6 PUFA	9.58	2.65	45.55
n-3/n-6	1.94	13.78	0.14

Values are mean of triplicate analysis.

[1] NFE = Nitrogen-free extract.

[2] -, not detected; SFA (saturated fatty acids) included 12:0, 14:0, 15:0, 16:0, 17:0 and 18:0; MUFA (monounsaturated fatty acids) included 16:1, 18:1 and 20:1; PUFA (polyunsaturated fatty acids) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6.

Diet composition

The proximate and fatty acid composition of experimental diets, containing graded levels of SSM are given in Table 3. The crude protein, crude lipid and digestible energy of the all experimental diets were of 38%, 10% and 15.75 kJ/g, respectively. The fatty acid composition SFA, MUFA and PUFA of the experimental diets ranged from 30.27 to 43.96%, 22.22 to 24.4% and 21.49 to 32.99%, respectively. Total n-3 of experimental diets followed the similar values as total n-6 fatty acids and ranged from 7.64 to 9.0% and 19.22 to 21.47%, respectively.

Growth performance

Carp fry fed graded SSM diets differed significantly in mean final weight, weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) (Table 4). The final weight of fish fed up to 75% SSM was significantly higher ($p<0.05$) than that of fish fed with the 100% SSM. This trend was significantly noticeable ($p<0.05$) for WG and SGR, in which there was obvious decreases as the SSM replacement approached 100%. FCR also significantly increased as the proportion of SSM was increased to 100% in the diet. No significant differences in survival were confirmed on the experimental groups ($p>0.05$).

Carcass proximate and fatty acid composition

The initial and final proximate and fatty acid compositions of fish fed the experimental diets containing

Table 3. The fatty acid (%, on total fatty acids) and proximate composition (%, on wet wt.) of experimental diets

Parameters	Graded levels of SSM in diets (%)				
	0 (Control)	25	50	75	100
Moisture	8.451±0.08	7.768±0.27	8.465±0.76	7.799±0.12	7.254±1.06
Crude protein	38.05±0.07	37.80±0.43	37.95±0.14	37.81±0.34	38.17±0.19
Crude lipid	9.738±0.39	9.910±0.34	10.09±0.67	10.30±0.44	9.920±0.34
Ash	12.27±0.06	12.52±0.47	11.72±0.17	10.82±0.00	11.06±0.18
Crude fibre	3.236±0.03	3.615±0.37	4.382±0.37	4.491±0.08	5.110±0.06
NFE[1]	28.23±0.29	28.37±1.04	27.39±1.08	28.76±0.07	28.47±1.02
DE (kJ g^{-1})[2]	15.74±12.2	15.77±11.2	15.73±6.52	15.98±8.59	15.87±23.9
Fatty acids[3]					
C14:0	4.06	3.70	2.99	3.54	3.95
C15:0	0.43	0.80	0.72	1.23	1.56
C16:0	28.80	24.00	18.98	18.7	20.16
C17:0	1.20	1.10	0.92	1.08	1.04
C18:0	9.47	7.70	6.66	6.05	5.35
C16:1n-7	4.04	4.40	4.65	5.93	6.72
C18:1 n-7	2.92	3.50	4.13	4.41	4.48
C18:1n-9	14.7	13.00	13.92	11.10	11.67
C20:1n-9	2.74	2.10	1.37	0.78	0.45
C18:2n-6	13.8	16.00	19.10	16.90	17.60
C18:3n-6	2.03	2.80	3.82	4.08	3.95
C20:4n-6	1.06	1.60	1.83	2.11	1.96
C18:3n-3	0.46	0.60	0.69	0.80	0.77
C20:5n-3	4.04	5.00	6.10	5.81	5.07
C22:5n-3	0.10	0.80	1.45	1.83	2.04
C22:6n-3	6.33	6.40	6.65	5.64	3.97
SFA	43.96	37.3	30.27	30.6	32.06
MUFA	24.4	23.0	24.07	22.22	23.32
PUFA	27.82	33.32	39.64	37.17	35.36
n-3PUFA	10.93	12.8	14.89	14.08	11.85
n-6PUFA	16.89	20.4	24.75	23.09	23.51
n-3/n-6	0.64	0.62	0.60	0.60	0.50

Values are mean (±SD) of triplicate analysis.

[1] NFE = Nitrogen-free extract. [2] Estimated using the factors 20.9 kJ g^{-1} protein, 37.7 kJ g^{-1} lipid and 14.6 kJ g^{-1} carbohydrate (NRC 1993).

[3] SFA (saturated fatty acids) included 14:0, 15:0, 16:0, 17:0 and 18:0; MUFA (monounsaturated fatty acids) included 16:1, 18:1 and 20:1; PUFA (polyunsaturated fatty acids) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6.

Table 4. Growth performance of carp fry (*Cyprinus carpio*) fed with experimental diets

Parameters[1]	Graded levels of SSM in diets (%)				
	0 (Control)	25	50	75	100
Survival (%)	100.0±0.00	100.0±0.00	98.66±2.30	98.66±2.30	97.33±4.61
IW (g)	0.296±0.00	0.290±0.00	0.301±0.00	0.296±0.00	0.298±0.00
FW (g)	6.177±0.15^{a}	6.156±0.03^{a}	6.142±0.00^{a}	6.135±0.36^{a}	5.562±0.22^{b}
WG (%)	1,985.5±10.43^{a}	2,021.6±50.55^{a}	1,939.7±52.37^{a}	1,967.2±98.31^{a}	1,764.3±39.31^{b}
SGR (% day^{-1})	3.375±0.00^{a}	3.394±0.02^{a}	3.350±0.02^{a}	3.364±0.05^{a}	3.250±0.02^{b}
FCR	1.404±0.01^{a}	1.412±0.00^{a}	1.420±0.00^{a}	1.414±0.05^{a}	1.582±0.04^{b}

Values are mean (±SD) of triplicate analysis.

$^{a-b}$ Values in the same row with the different superscripts are significantly different ($p<0.05$).

[1] IW = Initial weight; FW = Final weight; WG = Weight gain; SGR = Specific growth rate; FCR = Feed conversion ratio.

different proportions of SSM are shown in Table 5. No significant differences were determined for dry matter, protein, lipid and ash in the muscle of carp fry fed with graded SSM diets ($p>0.05$). Fatty acid analysis showed that SFA in fish muscle significantly decreased, but MUFA and PUFA were not changed with the increased dietary SSM.

Table 5. The fatty acid (%, on total fatty acids) and proximate (%, on dry wt.) composition of carp fry (*Cyprinus carpio*) fed the experimental diets

Parameters	Graded levels of SSM in diets (%)					
	Initial	0 (Control)	25	50	75	100
Dry matter	20.60	24.76±0.79	23.07±0.48	23.56±0.25	24.49±1.39	22.86±0.38
Crude protein	67.35	70.44±1.78	72.02±1.10	71.04±0.05	70.28±1.97	73.41±0.27
Crude lipid	17.13	18.32±2.60	14.85±0.88	15.45±0.66	16.94±2.59	13.72±0.95
Ash	15.51	12.22±0.82	13.3±0.15	13.49±0.72	12.77±0.62	12.99±0.17
Fatty acids [1]						
C14:0	2.99	3.37±0.18	3.02±0.25	2.12±0.32	2.73±0.29	2.42±0.98
C15:0	0.97	0.56±0.01^{bc}	0.46±0.12^{c}	0.79±0.02^{ab}	0.85±0.09^{a}	0.96±0.15^{a}
C16:0	24.2	20.73±0.41^{a}	20.59±0.91^{a}	20.52±0.67^{a}	17.95±0.77^{b}	19.95±0.14^{a}
C17:0	0.72	0.33±0.12^{c}	0.54±0.05^{bc}	0.77±0.04^{a}	0.63±0.11^{ab}	0.79±0.02^{a}
C18:0	9.36	7.72±1.03	6.72±0.94	7.9±0.09	6.02±0.48	7.49±0.91
C16:1n-7	4.47	4.49±0.96	4.69±0.26	4.41±0.48	5.37±0.33	4.73±1.08
C18:1n-7	3.61	3.57±0.30^{c}	3.54±0.12^{c}	4.31±0.02^{b}	4.64±0.09^{b}	5.11±0.09^{a}
C18:1n-9	17.89	18.72±1.92^{ab}	19.94±0.98^{a}	16.47±0.17^{b}	17.77±1.51^{ab}	16.42±0.41^{b}
C20:1n-9	1.84	2.31±0.06^{a}	1.80±0.14^{b}	1.61±0.14^{b}	1.52±0.02^{b}	1.02±0.17^{c}
C18:2n-6	6.89	18.32±1.01^{b}	23.02±1.49^{a}	18.16±1.23^{b}	21.98±2.71^{ab}	19.17±0.41^{ab}
C18:3n-6	0.86	2.32±0.19	2.94±0.22	2.68±0.205	3.24±0.30	2.72±0.60
C20:4n-6	3.47	1.96±0.13	1.76±0.28	2.37±0.36	2.07±0.44	3.2±1.10
C18:3n-3	0.3	0.92±0.17^{a}	0.34±0.05^{b}	0.29±0.01^{b}	0.38±0.00^{b}	0.2±0.07^{b}
C20:5n-3	4.34	3.015±0.30	3.08±0.11	3.41±0.51	3.16±0.25	3.22±0.60
C22:5n-3	0.94	0.55±0.07^{b}	0.47±0.03^{b}	1.19±0.00^{a}	1.23±0.24^{a}	1.60±0.23^{a}
C22:6n-3	10.25	5.88±1.39	5.08±0.52	6.38±0.17	4.44±0.87	5.69±1.34
SFA	38.24	32.71±1.11^{a}	31.33±2.29^{ab}	32.11±0.46^{a}	28.19±1.15^{b}	31.62±0.39^{a}
MUFA	27.81	29.10±3.25	29.97±1.27	26.81±0.53	29.31±1.78	27.29±1.76
PUFA	27.05	32.98±0.72	36.70±0.88	34.50±2.12	36.52±1.20	35.81±3.02
n-3 PUFA	15.83	10.37±1.80	8.97±0.54	11.28±0.31	9.22±0.37	10.71±2.11
n-6 PUFA	11.22	22.61±1.07^{c}	27.73±1.42^{a}	23.22±1.81^{bc}	27.3±2.57^{ab}	25.09±0.91^{abc}
n-3/n-6	1.410	0.46±0.10	0.32±0.03	0.48±0.02	0.34±0.08	0.42±0.06

Values are mean (±SD) of triplicate analysis.

$^{a-c}$ Values in the same row with the different superscripts are significantly different ($p<0.05$).

[1] SFA (saturated fatty acids) included 14:0, 15:0, 16:0, 17:0 and 18:0; MUFA (monounsaturated fatty acids) included 16:1, 18:1 and 20:1; PUFA (polyunsaturated fatty acids) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6.

Positive or negative changes in correlation with increased SSM could be observed also in various fatty acids. In particular, the amounts of 15:0, 17:0, 18:1n-7, 18:2n-6 and 22:5n-3 significantly increased, but 16:0, 18:1n-9, 18:3n-3 and 20:1 n-9 significantly decreased with increasing dietary SSM content. Total n-6 PUFA, improved with dietary SSM replacement, was found to be highest in muscle of fish fed the 25 or 75% SSM. Total n-3 PUFA, decreased as compared to initial fish, was not changed in muscle of fish fed with experimental diets. The ratio of n-3 PUFA to n-6 PUFA was not affected significantly in muscle of fish fed the experimental diets containing different proportions of SSM including control diet.

DISCUSSION

The present study showed that dietary SSM levels significantly affected the growth response of common carp fry. With increasing dietary SSM, growth significantly decreased ($p<0.05$). In replacements of FM protein over 75%, weight gain dropped significantly as compared to other experimental groups. However, no significant differences in weight gain were observed among fish fed the diets with less than 75% protein from SSM. SGR and FCR of mirror carp fry were also improved slightly when they were fed with the diet containing up to 75% SSM level without significant difference among them ($p>0.05$), but SGR and FCR decreased significantly in the case of 100% SSM replacement ($p<0.05$; Table 4). These results indicated that SSM could be used as a substitute for FM by up to 75% without any apparent negative effects on growth and feed utilization under the experimental diets in this study, and higher substitution levels resulted in growth reduction or poor feed utilization. This agreed well with the results of Gümüş et al. (2010) reported that no significant differences in growth performance of Nile tilapia fry (*Oreochromis niloticus*), fed with diets including up to 75% SSM. Abdelghany (2003) and Ahmad (2008) also found that 50% and 75% of the dietary FM protein could be replaced by gambusia (*Gambusia affinis)* fish meal protein in diets for fry of red tilapia (*O. niloticus*×*O. mossambicus)* and Nile tilapia (*O. niloticus*) with no significant differences in growth compared with FM control diet, respectively.

Previous studies showed that there were no significant differences in body composition (protein, lipid, ash and dry matter) among the fish fed the diets with graded levels of various animal protein sources (Yang et al., 2006; Ahmad, 2008; Hu et al., 2008; Gümüş et al., 2010). The dietary SSM in the present study did not significantly affect also the proximate body composition.

In this experiment, the distribution of fatty acid concentrations in muscle of fish for SSM has been clearly demonstrated in Table 5. In general, as FM was progressively replaced by SSM, some fatty acids inclined to increase or decrease in the muscle of fish. Our results show that the n-3:n-6 ratio in the muscle of fish progressively decreased as the SSM level increased in the diet, thus reflecting what occurs in the diet itself. The n-3 content of cultured fish has often been reported to be lower because of lowered fish meal usage in the diet (Pigott, 1989). Ackman (1967) found that the ratio of total n-3 to total n-6 acids was lower in the freshwater fish oils, suggesting a basic difference in dietary availability of these two groups of acids. However, carp and tilapia are generally regarded as omnivorous fish and are considered to require greater amounts of n-6 fatty acids than n-3 fatty acids in their diets (Takeuchi et al., 2002). High levels of n-3 have been reported to depress the growth of tilapia (Kanazawa et al., 1980; Ng et al., 2001) and carp (Du et al., 2008). However, it was observed that the level of n-3 PUFA didn't affect significantly fatty acid composition of fish fed with diets having SSM inclusions at different proportions. Similar results were reported in the Nile tilapia fed diets with graded levels of tuna liver meal (Gümüş and Erdogan, 2010).

With respect to the general fish fatty acid profile, MUFA tended to decrease (mainly due to 18:1n-9) as the SSM replaced increasingly FM. In addition, progressively higher SSM protein percentages also resulted in higher PUFA levels in the diet and the fish. This was due to an increasingly higher total content of n-6 fatty acids of the experimental fish fed diets including different proportion of SSM than that of the control diet. The freshwater origin of SSM could partially explain the source of these fatty acids. According to Watanabe et al. (1992) increasing the soybean meal content of the diet increases the levels of n-6 fatty acid in fish. In general, the differences in the studied diets had no significant impact on the fish fatty acid composition, as previously seen in certain studies in which the fatty acid levels were kept within precise limits, this indicates that the fatty acid composition is a characteristic of the species or tissue involved, and indeed it is relatively independent from the diet (Visentainer et al., 2005; Souza et al., 2007; Gümüş and Erdogan, 2010). Arzel et al. (1994) found a relatively constant fatty acid composition in the muscle lipids of *Salmo trutta* L. raised with different lipid sources. Similar results were also obtained by Rondan et al. (2009) in *Diplodus puntazzo* and Tonial et al. (2009) in *Oreochromis niloticus*.

The results of the present study indicate that dietary replacement of FM with SSM does not significantly modify carp fry fatty acid composition. Moreover, SSM has a favourable effect on the lipid composition in the muscle of fish. One positive effect of the diets based on SSM is the

increase in n-6 levels, the other is the constant n-3: n-6 ratio. Both are thought as important factors that affected the nutritional quality of fish muscle. Further research should be conducted on usage of SSM as a substitute of FM to determine acceptable fatty acid composition for large pond fish.

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