

Effect of soybean meal enriched diet on growth, body fatty acid composition and crude protein of *Mugil cephalus* fry

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Abstract – An experiment was conducted to investigate the effect of soybean meal enrichment on growth performance, protein content, fatty acid composition of *Mugil cephalus* fry (Mean initial body weight, 0.420 ± 0.06 g). Three experimental diets, based on 2 ingredients (an extruded diet (ED) and the soybean meal (SBM)), were formulated: control diet: D_c (50% ED and 50% SBM), D_{25} (75% of ED and 25% of SBM) and D75 (25% of ED and 75% of SBM). The results showed that high level of soybean meal affects negatively growth of *Mugil cephalus* fry specially at the second half of the experience. The protein content increase significantly, during experience, only for FD_c. We can then replace fish meal based diet with soybean meal until 50% for providing them with a good protein rate. Concerning lipid, major fatty acid is the 16:1. We find that unlike FD₇₅, FD₂₅ and FD_c total body fatty acids decreases significantly according to time. However, more the food is rich in soybean meal, more fatty acids, and particularly EPA and DHA levels decrease significantly at the beginning of the experiment. It seems that their proportion in diets D₂₅, D_c and D₇₅ is insufficient for providing a good fish growth.

Keywords: diet; fatty acids; growth; *Mugil cephalus*; protein; soybean meal.

Abreviations: Dc: Control diet; D25: Diet with 25% of soybean meal; D75: Diet with 75% of soybean meal; ED: Extruded diet; FA: Fatty acids; FDx: Fry fed with diet (x); HPUFA: High polyunsaturated fatty acids; SBM: Soybean meal; SPC: Soy protein concentrate; TAG: triacylglycerol; TFA: Total fatty acid

1. Introduction

In aquaculture, diet is a very important parameter that condition production and particularly fish production as well as in intensive or in semi-extensive production. Among the different constituent of fish diets, lipids are very determinant for growth and as being the favored form of non-protein metabolicenergy (Sargent et al. 2002). Lipids in fish diet come essentially from fish oil (FO) that is an invaluable dietary component for fish because it furnishes the essential fatty acids (EFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (AA) which are needed for optimal growth and development (Huang et al. 2007). Proteins diet also, are much important for fish because they contribute in metabolic energy. Moreover, proteins are the most important flesh constituent and the white fish flesh has a low proteosynthezis activity, so that fishes must find proteins in their diet and with a satisfactory amount. As lipids diet, proteins in fish diet come essentially from fish meal. However, because of the hay expensive cost of oil and meal fish as diet components, fabricants thought to replace them with a vegetable oil and meal source. In Tunisia, mullet fry are much used in stocking with fish the dam reservoirs and the hill lakes. Among the five mullet species existing in the Tunisian coasts, *Mugil cephalus* is considered that be the most important commercial one. And fry of this species show a great capacity adaptation to freshwater. Indeed, this small fishes are captured in the mouth wadi when they reach coasts after reproduction that was being in seawater. After that, they are directly transferred to continental freshwater. Furthermore, it was demonstrated that *Mugil cephalus* fry lose a significant amount of triacylglycerols (TAG) in consequence of passing in freshwater (khériji et al. 2003). This change of environmental medium involve, also, an increase of phosphatidilcholine (El cafsi, 2003), that



may be synthesized from TAG. So, we can suppose, that if we allow fry to pass by an acclimation time in which we give to them died enriched with phosphatidilcholine shaped soy lecithin, then we'll avoid the transformation of the TAG in to phosphatidilcholine. In this way, *Mugil cephalus* fry will prevent the loss of their energetic reserves when they rich freshwater medium. In order to test this hypothesis, and because of the non-existence of specific mullet diet, we have chosen to test the soybean meal as an ingredient in diet of *Mugil cephalus* fry and follow if this soy enriched diet have an influence on the fish lipid composition or not.

2. Materials and methodes

2.1. Biologic material

For our study, we have chosen mullet fry (*Mugil cephalus*) because it represents one of the much appreciated specie in Tunisian fishes. This specie is also, very widespread in Tunisian costs.

2.1.1. Fishs sampling and rearing

collect was made in the Khelij canal mouth situated in the Tunis gulf. This site offer tow advantages: accessibility and fry abundance. Fish was captured by means of an "Italian net" which mesh was of 1.5mm. As the sampling was made in a canal mouth, water salinity was variable. Fry caught at low water salinity was kept in freshwater medium. The ones caught in high water salinity, was kept in seawater. Freshwater fry was be placed in 200 and 400 liters aerated tanks, daily siphoned to eliminate feces and diet excess with a water renewal of one or two times a week according to water quality. Tanks was constantly aerated with a compressed air circuit (suppresser GAST Regenair R4110). They are exposed at daylight to provide them a natural photoperiod. Measurement of water salinity and temperature was made with a salinometer. Tap water Salinity is $1\pm 0,1$ g L-1 corresponding of the freshwater salinity in our study. Dissolved oxygen and pH was measured respectively with an oximeter and a pH-meter. Collected fry was very small (0.07g), so that, we need to make them bigger to be able to do later analysis. Therefore, we reared fry during eight months until they reach 0.41g as wet weight. After that we began our experience.

2.2. Experimental diet

The food used for our experience was a complete feeding stuff for fish witch is an extruded aliment from Aqualife, BioMar (tab. 1). It is composed of fish meal, oil seeds products and by-product, oils, cereal grain, vitamins and minerals. It was kindly furnished to us by Tunisian Aquaculture Society where they use it for sea-bream fish. We choose to mix it with soybean meal; we conclude that more the soy supplementation level is high more the fish meal level in the experiment diet is low.

composition	Additifs	
42%	Vitamin A (I.U./kg)	15000
22%	Vitamin D3 (I.U./kg)	3000
8,6%	Vitamin E (mg/kg)	210
2%		
1,23%		
2%		
	composition 42% 22% 8,6% 2% 1,23%	Additifs 42% Vitamin A (I.U./kg) 22% Vitamin D3 (I.U./kg) 8,6% Vitamin E (mg/kg) 2% 1,23%

Table 1: Composition of the extruded aliment

2.3. Fish treatment:

2.3.1. Preparation of Mugil cephalus fry diet

During this experience, we have placed about 26 fry per aquarium. We provide them with three different diets. Each diet was fed to duplicate. Diet preparation was simple because we have no data about specific mullet diet. We choose to mix the extruded sea-bream food with different proportions of soybean meal. Thus we have prepared:



* A control diet « Dc » formed with 50% extruded food and 50% of soybean meal. This diet was considered as a control one because we gave it to fish since their capture, this means, in the enlargement period.

* A first diet « D25 » formed with 75% of extruded food and 25% of soybean meal.

* A second diet « D75 » formed of 25% de extruded food and 75% of soybean meal.

The two later diets were furnished to fish only during the experience that remained eight weeks. Fish sampling (n=6, for lipid and protein analysis) were made for each diet, at the first, the fourth and the eighth week of the experience. Diet amount distributed to fish was 10% of their wet weight. At days, 12, 21, 29, 36 and 42 fry were weighted to adjust diet amount given to fish. Diet was distributed once a day, at the morning. We remarked that there is, almost, no food in excess. It's commonly adopted in this kind of experience, that before sampling, fish don't eat during 24 hours. In our case and in view of the fry small size, we choose to give aliment to fry until the day before the sampling to ovoid the minimum of fat lose. So, we proceed to eviscerate the fry in order to eliminate non digest and non-absorbed food from lipid and protein analysis.

2.3.2. Fatty acid composition analysis of Mugil cephalus fry:

2.3.2.1. Lipid extraction:

Lipid extraction has been realized according to Folch et al. (1957) method. Fatty acyl methyl esters were obtained according to Metcalfe and coll. (1966) using BF3, Boron trifluoride -methanol (14 %).

2.3.2.2. Fatty acid analysis by CPG

Individual FAMEs were separated and quantified by gas chromatography using a Hewlett-Packard, Palo Alto, CA Model 5890 equipped with a flame ionization detector (FID) and a column silica column HP-Innowax ($60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ µm}$) with nitrogen as the carrier gas at a flow rate of 1 mL/min; flame ionisation detection temperature 280°C; injector temperature 250°C; oven temperature programmed from 180 to 250 °C. Peaks were identified by comparison of their retention times with PUFA 3 FAMEs standards (SUPELCO).

2.3.3. Protein analysis of Mugil cephalus fry

Sampled fish was crushed in a phosphate tampon using an Ultra_turax. The broyat was centrifuged during 10 min at 9000 g. The protein analysis was carried out on the floating phase according to the Lowry et al. (1951) method.

2.3.4. Moisture content of Mugil cephalus fry

On the sampled fish, we do a spinal-cerebral section. After that, it was dried between to filter paper and weighted. We put it then into Steam room at 105°C during 24 hours.

2.4. Statistical analysis

Average comparison was done according to ANOVA analysis using the Duncun test.

3. Results and discussion

3.1. Diet effect on Mugil cephalus growth

At the beginning of the experience, fry eating diet D_{75} , highly enriched with soybean meal, have the best growing weight. At day 25, the three growing curves intersect showing that fry treated with the different aliments, reached the same wet weight. From this moment, we note a conversely situation, with the best growing weight for D_{25} treated fry (fig .1). We can suppose that during the first thirty days, fry found a new source of phosphatidilcholine brought by soybean meal, this can reduce the possible transformation of triacylglycerols to this phospholipidic class (El Cafsi, 2003). Then, and since they had reconstituted their fat reserves, it seems that they need no more important amount of phosphatidylcholine. At that time, it's the diet containing the low amount of soybean meal that offered the best fish growing.



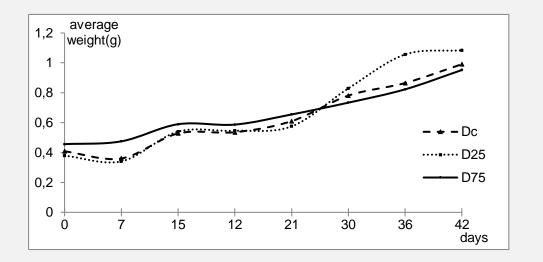


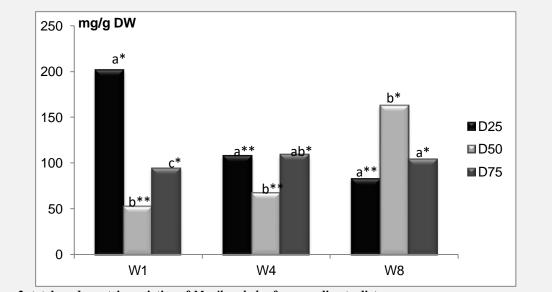
Figure 1: Growth weight (g) evolution of *Mugil cephalus* fry according to diet.

Discussion

Soy is used as one of the fish's food ingredients that done in substituting either oil or meal fish by oil or meal soy or soy protein concentrate (SPC). Most studies shows that inclusion, and particularly, high inclusion of soybean meal or SPC in fish diet have a negative effect on growth in comparison with many others protein source. This is valid for many species such Beluga (*Huso huso*) (Khajepour and Hosseini, 2012), salmonid fish (Collins et al. 2013).

The improvement of the quality of fish diet based on the by-product of soy seed, particularly soybean meal can be obtained by enrichment of this food by some additive such supplementation with amino acids in the aim of enhancing the fish growing rate. This is be demonstrated for juvenile *T. ovatus* when the soy diet contain methionine and lysine (Niu et al. 2015). For golden pompano, fish meal can be reduced to 240 g kg⁻¹, if it is substituted by SPC with 5 g kg⁻¹ taurine supplementation (Wu et al. 2015). For Nile tilapia larvae fed soybean meal-based diets, about 9.7 g kg⁻¹ dietary taurine is required for optimum performance (Al feky et al. 2015).

We can conclude that high level of soybean meal, in fish diet, affect negatively growth of fish and particularly *Mugil cephalus* fry, in our case.



3.2. Diet effect on *Mugil cephalus* body crude protein

Figure 2: total crude protein variation of *Mugil cephalus* **fry according to diet** (*The statistic test is done according to time (star mark: the difference between two values having equal number of star is not significant) and according to diet (letter mark: the difference between two values having a common character is not significant.)*



This figure shows that protein rate increases significantly during experience only for D_c treated fry. For D₂₅ treated fry, proteins decrease according to time and they fall significantly at the fourth and the eighth week with regard to the first one. However, D_{75} treated fry have a stable protein rate during all the experience and the small variation detected are not significant. Further to this experience, we can say that we can replace fish meal based diet with soybean meal until 50% for providing to fish a good protein rate. Also, we note that D_{25} which is mainly based on the extruded aliment used for sea-bream fish is not suitable for mullet fry because it did not allow them to have good protein content which significantly decrease at the end of the trial. Similar results were cited in the bibliography. In this context, Deng et al. (2006) showed that the apparent amino acid availability and protein efficiency ratio decreased with the increase of the CPS proportion in the food of juvenile Japanese flounder, Paralichthys olivaceus. The same result is obtained for Atlantic salmon (Mundheim et al. 2004). However, some others study shows that there is no effect of the substitution of fish meal by soybean meal or SPC on the fish protein metabolism (Elangovan and Shim, 2000). Our results are in concordance with bibliography and they can bring us to say that when the food is rich enough in lipids, proteins would be spared of the contribution in oxidative metabolism for the energy production. These proteins will then be digested and amino acids which arise from it will be used as precursors for the synthesis of novo body proteins. Our food rich in soybean meal, being so rather poor in lipid is going to direct fish to use food amino acids as energy source what is going to decrease the rate of protein accretion in various tissues of the animal and particularly the muscle.

3.3. Diet effect on Mugil cephlus total fatty acid content

According to time, we note the significant decrease of the TFA, by reaching the forth then the eighth week as well as in FD_c or FD₂₅. For FD₇₅, the decrease of the TFA according to time is not significant. At the first week of the trial, we register a significant increase of FD₂₅TFA, with regard to FD_c. On the other hand, the FD₇₅ TFA significantly decreased with regard to FD_c. We note also, although the difference is not significant, a low TFA content in FD₇₅ in comparison with FD₂₅ (fig.3). The effect of the variation of the food soymeal level enrichment on the TFA is felt essentially during the first week of trial, just after changing diet fry. Fry seems, afterward, to be acclimated with its new dietary food conditions.

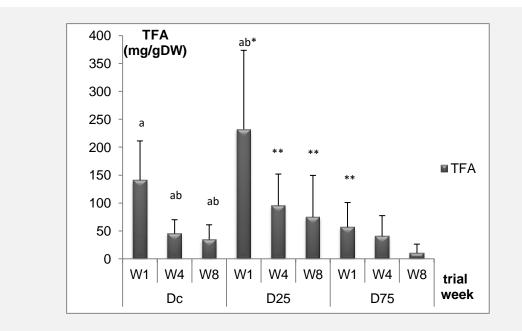


Figure 3: Total fatty acid variation (mg/gdw) in *Mugil cephalus* fry according to diet.

(The statistic test is done according to time (letter mark: the difference between two values having a common character is not significant) and according to diet (star mark: the difference between two values having equal number of star is not significant).



Discussion

In the first week of the experience FD₂₅ total fatty acid increase compared to FD_c seems to be due to the enrichment of the food in lipids; more the food is rich in soybean meal more its content in lipids is low and conversely. Indeed, the soybean meal used in the trial was a defatted soybean meal. Besides, FD₇₅ TFA decreased with regard to FD₂₅ and FD_c TFA because D_{75} is less rich in lipids than D_{25} and D_c . These results show that the content in TFA in our fishes reflects well that of the consummate food. On the other hand, we notice that the TFA decreased significantly according to time for FD_c and FD_{25} only. This can be due to the fact that before the starting up of the experience, fry are fed to satiation with two distributions a day. While during the experience, fry are fed at one time with a proportion of 10 % of the body weight. It is thus likely that this proportion is insufficient for fishes at this stage. For FD₇₅, there is no big variation of the TFA according to time. We can say that more the food is rich in soybean meal less the lipid reserves are exhausted according to time. This result seems to confirm our hypothesis, which supposes that the enrichment of the food in soybean meal and afterward, in soy lecithin so rich in phosphatidilcholine is going to help the fish to protect its lipid reserves since this last one is synthesized from triacylglyceroles (El Cafsi, 2003). In this context, the soya seed was used as ingredient in the fish food for under various forms: soy oil, soybean meal, soy protein concentrated. Other oleaginous seeds were also used such as the corn, the sunflower, the colza ... The extraction of the oil of these various seeds and its incorporation in the fish food was realized to substitute the fish oil and this, essentially, in an economic purpose because that cost much cheaper. However, the soybean meal and its protein concentrated were used as being a source of vegetable proteins which are also going to substitute animal proteins of aquatic origin (fish meal). Our study thus takes place between these two visions, because we tried to follow the effects of the incorporation of a defatted soybean meal on the lipid and protein fish composition. The majority of the studies show that the enrichment of fish diet with soybean meal or with SPC leads to the decrease of fish lipid content. (Messina et al. 2013). In this context, and to study the effect of the nature of the protein contribution on the lipogenesis and on the lipid accretion at Dicentrarchus labrax, results show that the daily fat gain is more important at fishes fed by the fish meal. On the other hand, levels of plasma triglycerides and cholesterol were lower in fish fed soy protein diets than in those fed the diet solely based on fish meal. This confirms our results because we found that the lipid reserves in our fishes decrease more the food is rich in soybean meal. The authors suppose that dietary protein sources affects fat deposition and the lipogenic potential in European seabass because the activity of some hepatic lipogenic enzymes such as the fatty acids synthetase decreases at fishes having received food rich in soy proteins (Dias et al. 2005). Jin et al. (2015), also, mentioned that fish fed with higher protein content diet was apt to have lower lipid content in muscle and liver. Food enrichment by soybean meal affects also cholesterol levels and plasma triacylglycerols in trout fish (Romarheim et al. 2008). The majority of the studies show well the negative effect of the incorporation of the soybean meal on the lipid content of fishes. This negative effect is due to the low content in lipid of this meal and not to the nature of the seed because the replacement of the fish oil by that of soya in the fish food can, according to certain authors, increase the lipid body content in to fishes. Indeed, juvenile seabream Acanthopagrus schlegeli, were fed four diets with ascending proportion replacement of fish oil by soybean oil. The study shows that the inclusion of soybean oil resulted in an increase in the whole body, the muscle and the liver crude lipid content (Peng et al. 2008). However, filets lipid content of the farmed Atlantic salmon fed with a Peruvian fish oil diet is slightly lower than those of fishes fed with the food containing the soybean oil (Bencze Rørå et al. 2005). Peng et al. (2014) explain that the increase of lipid deposition in the liver of turbot fed diets with higher dietary soy oil level would be related to the up-regulation of fatty acid synthesis-related gene (FAS) and the down-regulation of fatty acids oxidation gene (CPT I) expression.

3.4. Diet effect on the Mugil cephalus fatty acid composition

According to diet, showing the table four we notice, at first sight, that the fatty acid amount variations, due to the change of the diet, are significant only in the first week of the trial. We can say also, that in this time of experience, more the food is rich in soybean meal more we record a very important fall in the content of the various fatty acids with the exception of the stearic acid (18: 0) and the linolenic acid (18: 3) the contents of which are more important in fish fed the control diet (D_c) compared with FD₂₅. For the 18: 4ω 3, the content is more important in FD₇₅ diet. Any times, these exceptions are significant only for 18: 0. In the fourth week of the experience, the 16: 0, the 16: 1, 18: 2, 18: 4 and 20: 5 decreased at fry fed the control diet then they slightly increased at fry fed D₇₅. The content of 18: 3 is more



important in the control fry than to fry fed D_{25} and D_{75} . For all other fatty acids, we observed not significant decreases more the food is rich in soybean meal. In the eighth week, the content of 18: 3 and 18: 4 is a little more important in the control fry. For other fatty acids, more the food is rich in soybean meal more their contents are low. All these variations are not significant (tab.2).

	•		· ·	•	0		0		
	First week		Fourth week		k	Eighth week			
	D ₂₅	Dc	D ₇₅	D ₂₅	Dc	D ₇₅	\mathbf{D}_{25}	Dc	D ₇₅
C14 :0	^a 13,50	8,46	^b 3,85	^b 5,48	2,79	2,38	^b 6,88	2,48	0,78
C16 :0	^a 41,65	38,56*	^b 11,08 ^{**}	21,68	12,46**	13,04	^b 16,12	9,06**	2,94
C18 :0	^a 13,39	$16,29^{*}$	^b 2,71 ^{**}	^b 5,04	3,36**	2,64	^b 2,22	$1,67^{**}$	0,64
C16 :1	^a 15,91	^b 4,94	^b 2,49	^b 5,93	1,00	1,22	^b 5,65	2,42	0,58
C18 :1 ω9	^a 31,80	^b 16,82	^b 4,71	^b 14,64	5,39	3,59	^b 11,95	4,16	0,05
C18 :1 ω6	^a 5,48	4,38	^b 1,20	3,65	1,64	0,63	2,31	0,25	0,01
C20 :1	^a 12,83	^b 4,17	^b 1,10	^b 4,85	2,21	1,11	^b 4,00	1,04	0,57
C22 :1 ω9	^a 11,90	^b 1,66	^b 0,35	^b 3,01	0,48	0,18	^b 1,93	0,91	0,28
C22 :1 ω6	1,16	0,17	-	0,14	-	0,01	1,09	0,07	-
C24 :1	0,58	-	-	0,07	-	-	0,02	-	-
C18 :2 ω6	$25,15^{*}$	a19,53	8,61**	11,69**	7,34	7,87	7,76**	^b 4,06	1,92
C18 :3	3,89	5,02	4,32	1,29	2,20	1,90	1,72	2,25	0,41
C18 :4 ω3	4,87	4,17	^a 6,22	1,44	1,31	^b 1,79	1,44	1,83	^b 0,36
C20 :4	^b 1,56 [*]	^a 0,84	^b 0,02 ^{**}	0,47**	0,38	0,01	$0,52^{**}$	0,15	0,02
C20 :5 ω3	^b 15,15 [*]	a7,39	5,44**	5,36**	^b 1,82	2,28	4,18**	^b 0,91	1,28
C22 :5 ω3	^a 6,66	^b 1,21	^b 1,23	^b 1,88	0,73	0,42	^b 1,35	0,60	0,17
C22 :6 ω3	^a 26,71	^b 7,03	^b 3,22	^b 8,63	2,09	1,27	^b 5,75	2,66	0,23

Table 2: Fatty acid composition of Mugil cephalus fry according to diet and time (mg/g DW)

Table 3: Fatty acid composition of the Soybean meal and the extruded food.

FA 14 :0 16 :0 18 :0	SBM (mg/g WW) 0,03	Extruded food (mg/g ww) - - 0,29	SBM (%) 0,37 0,06	Extruded food (%) - - 2,21
16 :1 18 :1 ω9	0,11	0,57 0,33	1,33	4,1 2,19
18 :1 ω6 20 :1 22 :1 ω9	- 1,3 0.07	- 3,49 0,43	- 15,56 0,8	- 25,09 3,12
22 :1 ω6 24 :1	-	-	-	-
18 :2 \omega6 18 :3 18 :4 \omega3	1,14 0,04 0,44	0,57 0,05 0.94	13,6 0,48 5,25	4,08 0,4 6,78
20 :4 20 :5 ω3	- 4,46	- 5,63	- 53,17	- 40,43
22 :5 ω3 22 :6 ω 3	0,68 0,11	0,9 0,71	8,11 1,28	6,48 5,12

Discussion

These results are expected because we used for the preparation of our food, soybean meal among ingredients, the content of which in fat is low. For the docosahexaenoic acid (22:6 ω 3, DHA) and the eicosapentaenoic acid (20:5 ω 3, EPA), their contents are low all the more as the food is rich in soybean meal and it, during all the experience. Indeed, and in term of mass, the proportion of the ω 3HPUFA in diets D₂₅, D_c and D₇₅ are respectively: 0, 67 %, 0, 62 % and 0, 57 %. Therefore, more the food is rich in soybean meal less it contains the HPUFA. This explains the decrease of their fry contents in our case. FA composition of fry reflects well that of diets especially for EPA and DHA (tab.3). A close result was found in juvenile Channel Catfish *Ictalurus punctatus* (faukner et al. 2015). With regard to the bibliography, these contents turn out low and can affect the growth of fishes. It is what took place in our case because the growth of fry was lower at fishes fed with D_c and D₂₅ with regard to those fed with D₇₅ especially at the end of the dial. Regression of growth rate was observed also in juvenile European seabass fed low dietary n-3 HUFA (Skalli and Rubin, 2004) and, for the young black sea bream the



proportion of the HUFA ω 3 has to be more than 1 % (Peng et al. 2008). The same result was found for gilthead bream (Ibeas et al. 1996). It is possible, also, that our species presents a low power of enzymatic desaturation and elongation as it is the case of the young sea bream Sparus aurata (Mourente and Tocher, 1998) either that they present the symptom of deficiency in EFA (Castell et al. 1972) since the content of 18: 3 is rather low in the used food (tab.4). For 18:1 and 18:2, despite the high level of those fatty acids in the soymeal enriched diets, we observe that their content in FD_c and FD₇₅ is notably low. This result was mentioned in the Atlantic salmon, salmo salar, fed a sunflower enriched diet who showed that 18:2 $\omega 6$ and 18:1 $\omega 9$ are not preferentially retained in tissues even if the proportion of sunflower oil is more and more important (Matthew et al. 2003). For 18:4 while its content tends to be more important at extruded aliment based diet, its body rate is more important in fishes having received a proportion richer in soybean meal especially at the first and the fourth week. At the eighth week, its highest content is observed in FDc. The important decrease of the content of the majority of the HPUFA after the enrichment of the food in soybean meal is due to the fact that this last one is not rich in lipids. But, it seems that the nature of the seed also intervenes. That is the specter of fatty acids characterizing the soya seed is certainly different from that characterizing the fish oil. Subsequently, the fatty acid body composition in fishes fed with fish oil based diet is going to be different from that of fishes fed with a food with soya oil. This is confirmed by numerous works the majority of them underlined the decrease of the HUFA, and particularly, the EPA and the DHA, and the increase of linoleic $\omega 6$ and linolenic $\omega 3$ acids to fishes fed with the soya oil or the other oleaginous seed with regard to those fed by the fish oil. This was observed in young black sea bream (Peng et al. 2008). Same results are also obtained with fish oil substitution by other oleaginous seed such oil of canola and linseed at the trout Oncorhynchus mikyss, (Drew et al. 2007).

According to time, we note globally, the decrease of the majority of the fatty acids and this for the three diets. Some registered increases, are not significant.

To first at the fourth experience week, we note for fry fed with D_{25} , a significant decrease of , 18 :0, 16 :1, 20 :1, 22 :1w9, 18 :3, 18 :4, 20 :4, 20 :5, 22 :5, 22 :6. At the eighth week, fatty acids continue their decrease witch become significant with regard to the first week for this fatty acids: 16 :0, 18 :0, 16 :1, 20 :1, 22 :1 ω 9, 18 :2, 18 :4, 20 :5, 22 :5, 22:6. For fry fed with D_c , we note a significant decrease of 14 :0, 16 :0, 16 :1,18 :1 ω 9, 18 :2, 20 :5. At the eighth week decrease is significant with regard to the first week for: 14 :0,16 :0, 18 :0, 18 :1 ω 9, 18 :1 ω 6, 18 :2, 20 :5. For D_{75} treated fry, significant fatty acid decease is noted for, 18 :4, 20 :5, 22 :5 at the fourth and the eighth week with regard to the first one.

Discussion

In spite of the wealth in lipid of D_{25} with regard to D_c , we notice for fry fed with D_{25} , at the end of the fourth week, there was a content decrease of a more important number of fatty acids according to time, with regard to fry fed with D_c . The same result is registered in the eighth week. On the other hand, and for the D_{75} , globally we did not register significant variation of the content of various fatty acids according to time. In other words, the more the food is rich in soya, the more the fatty acid contents are stable in time. Thus, when the food is rich in lipids the fish uses its lipid reserves as energy source, especially as the distributed quantity turns out insufficient. When the food is rich in soybean meal the fish keeps, on the contrary, his pool of fatty acids according to time. We can have two hypotheses which can explain this result. The already quoted hypothesis is that the enrichment of the food in soy lecithin so rich in phosphatidylcholine slows down the catabolism of the TAG Intended to the synthesis of the phosphatidylcholine (El Cafsi, 2003). The second is that the soybean meal constitutes for the animal an important source of proteins. It is thus possible that fishes are going to use proteins as source of energy rather than lipids because, to fishes, generally, and with regard to mammals, a bigger part of the AA brought by the food enter the oxidative metabolism (Guillaume et al. 2006).

4. Conclusion

In conclusion, we demonstrate that that fish food enrichment with soybean meal is benefic for growth only in court term. After that, a growth decrease was observed in mullet fry. Then this enrichment must be only envisaged in short period after fry transfer from sea to freshwater. As for the body proteins content, we conclude that either high and low enrichment by soybean meal in fish food didn't increase the fry total body protein content. Only fish fed control diet (average amount of soybean meal) present



the best rate of proteins because they increase according to time in *Mugil cephalus* fry. Whereas high proportions assure their stability while the low proportions of soybean meal affect them negatively.

Finally, and concerning fry lipid composition, we noticed that the total fatty acids decreases more the food is rich in soybean meal. This result is expected because the meal was obtained after grinding and extraction of the oil. Of or, the enrichment of the food with soybean meal means its impoverishment in lipids, so, the content in TFA in our fishes reflected well that of the consummate food. However, we also observed that it's the highly enriched food in soybean meal that protects best the total fry fatty acids according to time. On the other hand, for the two other foods, this content decreases during the experiment. As regards the fatty acids, particularly at the beginning of the experiment, further to the enrichment of the food in soybean meal. This is due to the decrease of the quantity of lipids in the enriched food. If we examine the HPUFA and particularly the EPA and the DHA, we notice that their contents also decreased as almost all other fatty acids. These contents with which we proceeded seem to be insufficient and can affect the mullet fry growth.

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6. Références

- Al-Feky SSA, El-Sayed AFM, Ezzat AA (2015) Dietary taurine enhances growth and feed utilization in larval Nile tilapia (*Oreochromis niloticus*) fed soybean meal-based diets. Aquaculture Nutrition. doi:10.1111/anu.12266
- Bencze Rørå AM, Sveinung B, Lisbeth H, Turid R, Torstein S, Bjørn B (2005) Quality characteristics of farmed Atlantic salmon (*Salmo salar*) fed diets high in soybean or fish oil as affected by cold-smoking temperature *LWT Food Science and Technology* 38: 3, 201-211.
- **Castell D, Sinnhuber RO, Wales JH, Lee JD** (1972) Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. J. Nutr. 102: 77–86.
- **Collins SA, Øverland M, Skrede AD, Drew MD (2013)** Effect of plant protein sources on growth rate in salmonids: Meta-analysis of dietary inclusion of soybean, pea and canoa/rapeseed meals and protein concentrates. *Aquaculture* 400-401, 85-100.
- **Deng J, Mai K, Aia Q, Zhanga W, Wanga X, Xua W, Liufua Z (2006)** Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 258: 1-4, 503-513.
- **Deng J, Mai K, Ai Q, Zhang W, Wang X, Xu W, Liufu Z (2006)** Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* 258: 1-4, 503-513.
- **Drew MD, Ogunkoya AE, Janz DM, Van Kessel AG (2007)** Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout (Oncorhynchus mykiss). *Aquaculture*. 267: 260–268.
- **Elangovan A, Shim KF (2000)** The influence of replacing fish meal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (*Barbodes altus*). *Aquaculture*, 189: 1-2, 133-144.
- El Cafsi M (1998) Effet de la salinité du milieu sur les lipides des tissus musculaires de *Liza aurata* (Risso,1810). *Ichtyophysiol. Acta*, 21 : 15-25.
- El Cafsi M, Romdhane MS, Chaouch A, Masmoudi W, Khériji S, Chanussot F, Chérif A (2003) Qualitative needs of lipids by mullet, *Mugil cephalus*, fry during freshwater acclimation. *Aquaculture*, 225: 1–4, 233-241.
- **Faukner J, Rawles SD, Sink TD, Lochmann R, Proctor A, Chen R, Phillips H** (2015) The Effects of Diets Containing Standard Soybean Oil, Soybean Oil Enhanced with Conjugated Linoleic Acids, Menhaden Fish Oil, or an Algal Docosahexaenoic Acid Supplement on Juvenile Channel Catfish Performance, Hematology, Body Composition, and Nonspecific Immune Response. North American Journal of Aquaculture, 77 : 2, 217-229.



- Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.
- Guillaume J, Kaushik S, Bergot P, Metailler R (2006) Nutrition et alimentation des poissons et crustacés. Edition INRA-Ifremer, p.489.
- Huang SSY, Oo AN, Higgs DA, Brauner CJ, Satoh S (2007) Effect of dietary canola oil level on the growth performance and fatty acid composition of juvenile red sea bream, Pagrus major. *Aquaculture*. 271: 420–431.
- **Ibeas C, Cejas J, Gomez T, Jerez S, Lorenzo A (1996)** Influence of dietary n 3 highly unsaturated fatty acids levels on juvenile gilthead seabream (*Sparus aurata*) growth and tissue fatty acid composition. *Aquaculture* 142: 221–235.
- Jin Y, Tiana LX, Xie SW, Guo DG, Yang HJ, Liang GY, Liu YJ (2015) Interactions between dietary protein levels, growth performance, feed utilization, gene expression and metabolic products in juvenile grass carp (*Ctenopharyngodon idella*). Aquaculture. 437 : 75–83.
- **Khajepour F, Hosseini SA (2012)** Citric acid improves growth performance and phosphorus digestibility in Beluga (Huso huso) fed diets where soybean meal partly replaced fish meal. *Animal Feed Science and Technology*. 171, 68–73.
- Khériji S, El Cafsi M, Masmoudi W, Castell JD, Romdhane MS (2003) Salinity and Temperature Effects on the Lipid Composition of Mullet Sea Fry (*Mugil cephalus*, Linne, 1758). Aquaculture International, 11; 6, 571-582.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.
- Matthew PB, Chris GC, Peter DN (2003) Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comparative Biochemistry and Physiology*. Part B 135: 611-625.
- Messina M, Piccolo G, Tulli F, Messina CM, Cardinaletti G, Tibaldi E (2013) Lipid composition and metabolism of European sea bass (Dicentrarchus labrax L.) fed diets containing wheat gluten and legume meals as substitutes for fish meal. *Aquaculture*. 376-379: 6–14.
- Metcalfe LD, Schmitz AA, Pelka JR (1996) Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. *Ann. Chem.*, 38: 524-535.
- Mourente G, Tocher DR (1998) The in vivo incorporation and metabolism of [1-¹⁴C] linolenate (18:3n-3) in liver, brain and eyes of juveniles of rainbow trout *Oncorhynchus mykiss* L and gilthead sea bream *Sparus aurata* L., *Fish Physiol. Biochem.* 12: 2, 149–165.
- Mundheim H, Aksnes A, Hope B (2004) Growth, feed efficiency and digestibility in salmon (*Salmo salar* L.) fed different dietary proportions of vegetable protein sources in combination with two fish meal qualities. *Aquaculture*, 237: 1-4, 315-331.
- Niu J, Figueiredo-Silva C, Dong Y, Yue YR, Lin HZ, Wang J, Wang Y, Huang Z, Xia DM, Lu X (2015) Effect of replacing fish meal with soybean meal and of DL-methionine or lysine supplementation in pelleted diets on growth and nutrient utilization of juvenile golden pompano (*Trachinotus ovatus*). Aquaculture Nutrition, 21: 2.
- **Peng S, Chen L, Qin JG, Hou J, Yu N, Long Z, Ye j, Sun X (2008)** Effects of replacement of dietary fish oil by soybean oil on growth performance and liver biochemical composition in juvenile black seabream, *Acanthopagrus schlegeli Aquaculture* 276: 1-4, 154-161.
- Peng M, Xu W, Mai K, Zhou H, Zhang Y, Liufu Z, Zhang K, Ai Q (2014) Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (Scophthalmus maximus L.) fed diets with various fish oil substitution levels by soybean oil. Aquaculture 433: 442–449.
- **Romarheim OH, Anders S, Michael P, Liv TM, Åshild K, Trond S (2008)** Lipid digestibility, bile drainage and development of morphological intestinal changes in rainbow trout (*Oncorhynchus mykiss*) fed diets containing defatted soybean meal. *Aquaculture*, 274: 2-4, 329-338.
- Sargent JR, Tocher DR, Bell JB (2002) The lipids, chapter 4. J.E. Halver, R.W. Hardy (Eds.), Fish Nutrition (3rd ed.), Academic Press, San Diego.
- Skalli A, Robin JH (2004) Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (Dicentrarchus labrax) juveniles: growth and fatty acid composition. Aquaculture. 240: 399– 415.